

## Molecular sequences derived from Paleocene Fort Union Formation coals vs. associated produced waters: Implications for CBM regeneration

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### ABSTRACT

Coalbed methane regeneration is of increasing interest, and is gaining global attention with respect to enhancement of gas recovery. The objective of this study is to determine if there are differences in methanogen nucleic acid sequences associated with low rank coals from the Powder River Basin, Wyoming, in comparison with sequences that can be recovered from coal bed-associated produced waters. Based on results obtained to date, the sequences from the coals appear to be associated with putatively deep-rooted thermophilic autotrophic methanogens, whereas the sequences from the waters are associated with thermophilic autotrophic and heterotrophic methanogens. The recovered sequences associated with coal thus appear to be both phylogenetically and functionally distinct from those that are more closely associated with the produced water. To be able to relate such recovered sequences to organisms that might be present and possibly active in these environments, it is suggested that direct observation, followed by isolation and single cell-based physiological/molecular analyses, be used to characterize methanogenic consortia possibly associated with coals and/or produced waters. It is also important to characterize the microenvironment where these microbes might be found, in both ecological and geological contexts, to be able to develop effective, ecologically relevant coalbed methane regeneration processes.

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## 1. Introduction

### 1.1. The historical context for coalbed methane generation

The process of methanogenesis occurs in a wide range of environments (Jaing et al., 2003; Etiope et al., 2004; Tao et al., 2007), resulting in direct methane releases to the atmosphere, accumulation of methane in a wide variety of geological structures, and accumulation of methane hydrates in deep marine environments and tundra areas as major examples. Much of this methane is present in subsurface waters that have relatively long residence times. As an example, fluids in Paleocene–Eocene coal beds of the northern Gulf of Mexico (McIntosh et al., 2007) have a residence time >16 ka. In coal basins (Slater et al., 2006), methane generally has no detectable <sup>14</sup>C activity; this can be related to long hydrologic residence times and/or the absence of modern carbon. The potential for methane recovery from coal beds occurs world-wide (Rice and Nuccio, 2000); this has led to the development of coalbed methane (CBM) as an energy resource, such as in the Powder River Basin (PRB) in Wyoming, USA (Fig. 1). The source of this methane, particularly when it is associated with coal

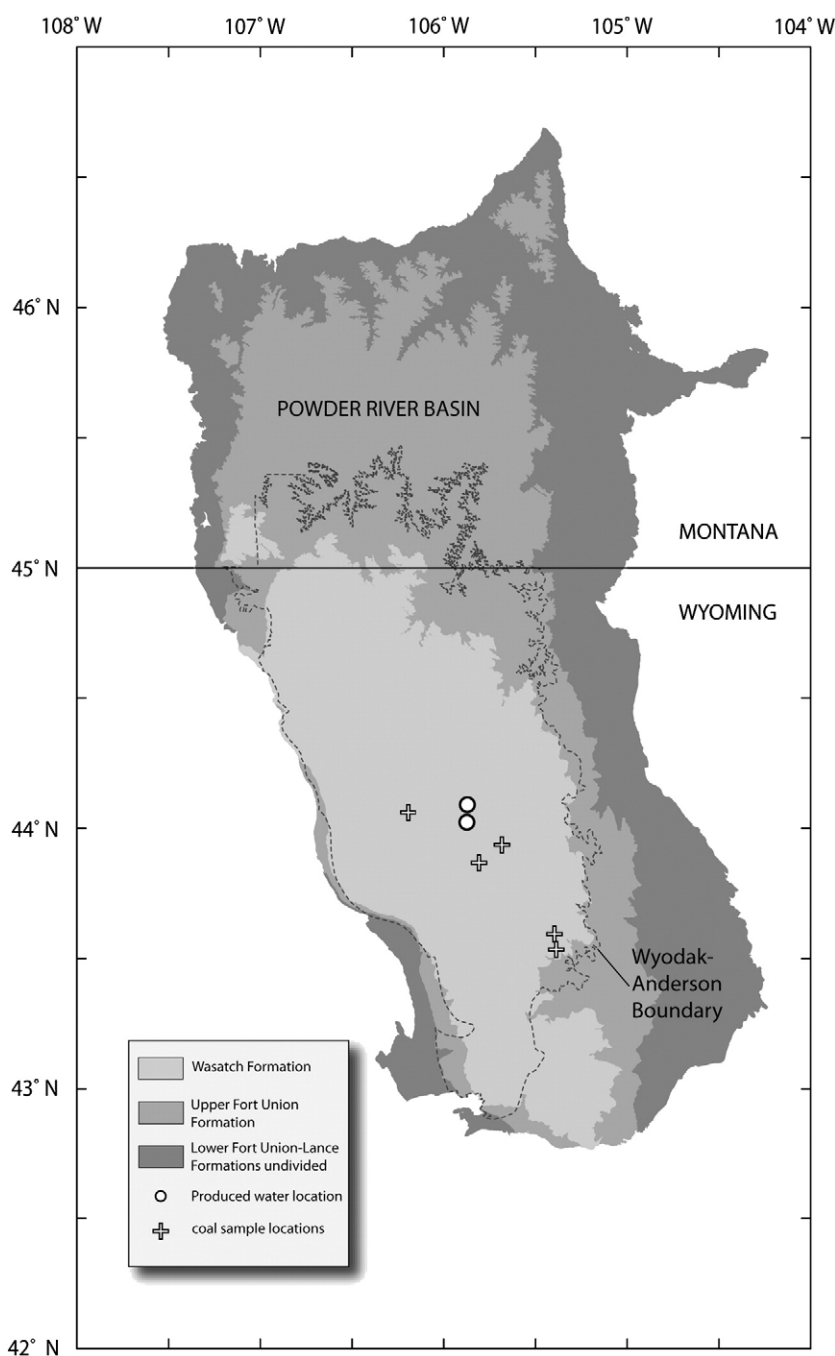
and organic-rich shale, is of widespread geological and economic significance (Flores et al., 2007), and is of added importance in the context of developing effective, realistic CBM regeneration strategies. An extended period of time is required to accumulate a CBM resource. For example, it is estimated that methane associated with PRB coals accumulated during the period 65 Ma to 2 Ma (Flores et al., this volume). However, this timing may be best constrained by age tracers (carbon-14, noble gases, and tritium) coupled with stable isotopes (C,H,O), and elemental chemistry (anions, cations, nutrients). Whether viable archaeans, including methanogens, were present in coal-bearing rocks, their source and possible functioning under contemporary conditions is of particular interest. These coals may provide conditions that would allow survival of these organisms since the time of coal formation.

Based on studies of other long-isolated environments, microbes may be able to survive for long periods. Reports of microbes having been cultured from 250-Ma salt crystals (Satterfield et al., 2005) and from 120-Ma and 35- to 25-Ma amber (Lambert et al., 1998; Greenblatt et al., 1999) are available. Methanogens also have been recovered from coals and their associated waters by classic cultural techniques (Falz et al., 1999).

During the past few decades, the role of methanogens in the formation of CBM, including the application of stable isotope analyses

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**Fig. 1.** The Powder River Basin of Wyoming and Montana showing sample locations used for microbe and produced water studies. See Table 1 for sample locations.

( $^{13}\text{C}/^{12}\text{C}$ ,  $^{14}\text{C}/^{12}\text{C}$  ratios) and  $^2\text{H}/^1\text{H}$  analyses, has provided valuable information on the temporal aspects of methane generation and accumulation in coal beds as well as in organic-rich shales (Whiticar, 1999; Slater et al., 2006; Strapoć et al., 2007). This is of particular interest in terms of fostering a better understanding of the generation and replenishment of natural gas resources. Biogenic gas from microbial metabolism has emerged as a significant resource in subbituminous coal beds in the PRB and other sedimentary basins around the world. In the PRB, methane has been characterized in part as acetoclastic in origin, associated with the movement of meteoric water from basin margins where exposed coal beds and overlying rocks, commonly have been burned and baked into clinkers (Heffern and Coates, 1999; Flores et al., this volume). In addition, biogenic methane from bacterial reduction of  $\text{CO}_2$  may have played a major role

in generating gas in the PRB and other coal basins (Rice, 1993; Gorody, 1999; Flores et al., this volume). This is particularly important in the deeper part of coal basins where biogenic gas has mixed with thermogenic gas in higher rank coal beds. The source(s) of methane in such mixtures can be estimated based on  $^{13}\text{C}/^{12}\text{C}$  ratios (Scott et al., 1994).

## 1.2. Approaches to study of CBM microbial assemblages

### 1.2.1. Cultural techniques using laboratory-based studies

Most efforts to understand the role of contemporary microbes in CBM regeneration have involved laboratory-based incubation studies, using coal samples collected from drillholes and from co-produced waters. In many cases, the specific source of the produced waters in

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