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Syntrophics Bridging the Gap of Methanogenesis in the Jharia Coal Bed Basin

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Abstract

The bituminous and sub-bituminous rank of coals is being produced from the Jharia basin of Jharkhand which is the largest producer of CBM in India. Although there have been many reports on methanogenesis from Jharia, the present study deals with the special emphasis on the syntrophic microbes which can act as catalyst for the hydrogenotrophic methanogenesis. Using the metagenomic approach followed by 454 pyro sequencing, the presence of syntrophic community has been deciphered for the first time from the formation water samples of Jharia coal bed basin. The taxonomic assignment of unassembled clean metagenomic sequences was performed using BLASTX against the GenBank database through MG-RAST server. The class clostridia revealed a sequence affiliation to family Syntrophomonadaceae and class Deltaproteobacteria to family Desulfobacteraceae, Pelobacteraceae, Syntrophaceae, and Syntrophobacteraceae. Results revealed the possibility of thermobiogenic methanogenesis in the coal bed due to the presence of syntrophs related to Syntrophothermus genus. The presence of such communities can aid in biotransformation of coal to methane leading to enhanced energy production.

Keywords: Coal bed methane; Metagenomics; Syntrophics; Hydrogenotrophic methanogenesis

Introduction

Coal is a pre-dominant source of energy in India, coming both from surface (70%) and underground mining (30%) activities. However in India, underground coal extraction leads to pollution due to inefficient mining techniques. In this context, underground coal gasification and coal bed methane are two left options which require much less surface area than traditional coal mining and therefore representing potential methods to extract energy from deep and isolated coal and lignite deposits. Coal has been considered as an unconventional natural gas reservoir where both thermogenic and biogenic methane is stored predominantly in an adsorbed state. Considering the vast worldwide reserves of coal, the ever-expanding need for energy across the globe, and the environmental benefits that focus on methane utilization as an energy source, there is considerable interest in stimulating the modern bioconversion of coal to methane. Coal is a heterogeneous organic material only chemically characterized [1,2]. Due to its inherent chemical complexity and recalcitrance, coal remains far from being an ideal substrate for methanogenic fermentation processes. However, it can reasonably be thought that, like other forms of organic matter, the anaerobic mineralization of coal will require a nutritionally diverse assemblage of anaerobic microbes that attack large molecular weight components and transform them to simpler chemical residues. The latter components ultimately get transformed to a set of volatile fatty acid (VFA) and alcohol intermediates. In particular, the oxidations of reduced organic compounds such as acetate, propionate, butyrate and benzoate require extremely low product concentrations. The concentrations of the products such as H_2, formate carbon dioxide, methanol and acetate are kept low by methanogenic archaea during methanogenic decomposition. Such symbiotic relationships, in which the two partners involved depend on each other for available metabolic energy, are called syntrophic interactions [3,4]. Wetlands, freshwater sediments, landfills and digestive tracts of animals are examples of such habitats, but importance of syntrophism in biogenic coal bed methane generation in India have not been elucidated. Hence, in this study attempts have been made for the first time to observe the coverage of syntrophs occurrence in formation water and coal sample via pyro sequencing study. The direct metagenomic analysis of coal sample from mine and CBM formation water provides the option to observe the coverage of syntrophs, as the approach will help in depicting the clear picture rather than culture based and cloning based studies.

Materials and Methods

Sampling

Formation water samples (approximately 200 L) were collected from the CBM well methane producing site of the West Jharia Coal Basin, Moonidih Coal Mines, Bharat Coking Coal Limited (BCCL), Jharkhand, India having depth of 700 m. The water samples were collected in pre-autoclaved bottles containing 0.5% resazurin as a redox indicator, Cysteine-HCl, Na-Thioglycolate for generating anaerobic condition. The bottles were filled completely during the sample collection to remove any gas from the head space and were sealed with crimp seal. Care was taken to avoid any air in the head space while sealing with crimp seal. The bottles were then taken to laboratory in ice box and the subsequent DNA extraction was performed within 24 h after collection. Formation water sample from the respective regions are the visual representation of the samples from that area.

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DNA extraction, sequencing and analysis

In recent times, the outburst of community genomics, or metagenomics, where the genetic material is directly sequenced from the environmental samples has offered fruitful insight into microbial population. DNA is sequenced without cloning, using one of the so-called next-generation sequencing techniques, usually pyro sequencing. Despite of the errors biased towards certain mistakes, this technique has comparatively higher throughput as well as lesser error rate per base sequenced than the Sanger sequencing [5]. A minimum of 5 µg of environmental DNA is needed for 454 pyro sequencing and to exclude the extraction bias [6].

The collected formation water samples were subjected to vacuum filtration using 0.22 µm, 47 mm diameter, (Millipore, USA) filter papers. The collected filter papers were subjected to DNA extraction using PowerWater DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) as recommended by the manufacturer.

The quantification of the extracted DNA was performed using NanoDrop spectrophotometer and the quality of the DNA was checked through gel electrophoresis. Samples were also analysed on Agilent 2100 Bioanalyser for the required quality and quantity check for undergoing pyro sequencing. Metagenomic whole genome sequencing was performed on 454 GS FLX (Roche Applied Science, USA) at NexGenBio Life Sciences, New Delhi.

Taxonomic assignment of the metagenomic reads

Metagenomic sequences obtained from the 454 GS FLX of an average size of 563 ± 178 bp for Formation Water (FW) were analysed using MG-RAST. The taxonomic assignment of unassembled clean metagenomic sequences was performed using BLASTX against the GenBank database through MG-RAST server. Best hit classification was used for determining the genus with maximum e-value cut off having $1e^{-5}$, minimum identity of 60% and minimum alignment length cut off having 45. The presence of particular syntrophic communities was considered on the basis of presence of housekeeping genes such as rpo and ssu [7]. The publicly accessible MG-RAST accession codes generated for FW was 4525867.3.

Results and Discussion

Syntrophism is a unique case of symbiotic assistance between two metabolically diverse microbes which depend on each other for degradation of a certain substrate, typically for energetic reasons. The syntrophic reaction carried out by certain anaerobic microbes facilitates the transfer of H_2 to hydrogen utilizing partner such as acetogens and methanogens. This transfer requires the continuous uptake of hydrogen by hydrogenotrophic methanogens or acetogens in order to prevent an increase of the partial pressure of hydrogen [8].

The correspondence of sequences with the family within the metagenome has been studied (Table 1). The obtained information suggests the possibility of presence of the syntrophic communities listed in the (Table 1). The class clostridia reveal a sequence affiliation to family Syntrophomonadaceae with an average e value of -23.67 and -21.87 and abundance of 70 and 74 for genus Syntrophothermus and Syntrophomonas respectively.

For the class Deltaproteobacteria (Table 1), the corresponding sequences were assigned to family Desulfobacteraceae, Pelobacteraceae, Syntrophaceae, and Syntrophobacteraceae and genus Desulfatibacillum, Pelobacter, Syntrophus, Syntrophobacter with an average e value of -23.52, -25.90, -28.73, -23.75 and abundance of 246, 497, 308, and 283 respectively in the metagenome.

The biomarker study suggested the probable presence of syntrophic genus on the basis of homology with housekeeping genes such as rpo and ssu (Table 2). From the genus Syntrophomonas and Syntrophothermus, a putative presence of *Syntrophomonas wolfei*, *Syntrophothermus lipocalidus* may be obtained in the environment. *Syntrophomonas wolfei* under the genus Syntrophomonas is chemoorganotrophic microbe growing in mesophilic conditions and anaerobically β-oxidizes fatty acids through protons serving as the electron acceptor. Substrates such as butyrate, caproate, and caprylate are degraded to acetate and H_2 whereas valerate and heptanoate are degraded to acetate, propionate and H_2 [9]. Hence, the probable presence of this genus Syntrophomonas in the studied metagenome indicates the probable degradation of the volatile fatty acids via the β- oxidation. Reports suggest the growth of *S. wolfei* is restricted by carbohydrates, proteinaceous materials, alcohols, or other organic compounds [9]. Fatty acid degradation take place only in syntrophic association with H_2-utilizing bacteria and are stimulated by factors in rumen fluid and/or B vitamin mixture. This microbe has been found from anaerobic environments, such as aquatic sediments, sewage digester sludge where organic matter is degraded yielding CO_2 and CH_4 as major products.

Syntrophothermus genus having *Syntrophothermus lipocalidus* utilizes saturated fatty acid with 4 to 10 carbon atoms by β-oxidation in syntrophic association with hydrogenotrophic methanogens [10]. This microbe grows in an environment having optimum pH of 6.5-7.0 and temperature of 55°C. The sequence correspondence through biomarker homology to Syntrophothermus suggests the possible catabolism of the saturated fatty acid (portion of the coal organics) in syntrophic union with the hydrogenotrophic methanogen in thermophilic conditions.

Sequence affiliation of the genus Syntrophobacter to ssu (average e value = -20) has been observed in the metagenome. *Syntrophobacter fumaroxidans* a member of genus Syntrophothermus is a neutral, mesophilic fumarate oxidizing microbe. It is strictly anaerobic and specifically grows syntrophically on propionate with the hydrogenotrophic methanogens such as *M. hungatei* and ferments fumarate, malate, aspartate and pyruvate. Presence of such genus in the metagenome suggests a possible role in formate transfer as per the report by de Bok et al. [11] in the co-culture of *S. fumaroxidans* with *M. hungatei* syntrophically. Along with sulphate, thiosulphate and fumarate can also serve as electron acceptors, however nitrate is not reduced. It can also be isolated from granular sludge of an up flow anaerobic sludge bed reactor treating sugar-beet processing wastewater [12]. The sequence affiliations to this genus indicate the syntrophic activity present in the Jharia subsurface microbiome. This syntrophic activity helps in collection of the substrate of the hydrogenotrophic methanogenesis.

Anaerobic digestion of organic matter results formation of acetate which is the main precursor for methane formation. Methane formation takes place either by hydrogenotrophic or aceticlastic pathway. Methanogens operating in aceticlastic pathway are of family Methanosarcinaceae or Methanosaetaceae. The former one has the higher growth rate and threshold for acetate [13]. The hydrogenotrophic pathway operates in two step in which acetate is oxidized to H_2 and CO_2, and later the oxidized products combine to form methane [14]. The insight of the hydrogenotrophic pathway has been obtained in the earlier studies reported from Jharia coal bed basin [15]. This reaction is carried out by acetate oxidizing bacteria (often *Clostridium* sp.) in syntrophic association with hydrogenotrophic methanogens such as Methanomicrobiales or Methanobacteriales [16-18]. This study reveals

Phylum	Class	Family	Genus	Average e- value	Abundance
Firmicutes	Clostridia	Syntrophomonadaceae	Syntrophomonas	-23.6	70
Firmicutes	Clostridia	Syntrophomonadaceae	Syntrophomonas	-21.87	74
Proteobacteria	Delta proteobacteria	Desulfobacteraceae	Desulfatibacillum	-23.52	246
Proteobacteria	Delta proteobacteria	Pelobacteraceae	Pelobacter	-25.90	497
Proteobacteria	Delta proteobacteria	Syntrophaceae	Syntrophus	-28.73	308
Proteobacteria	Delta proteobacteria	Syntrophobacteraceae	Syntrophobacter	-23.75	283

Table 1: The plausible presence of the syntrophic communities in the subsurface microbiome of Jharia coal bed basin.

S. No.	Genus	Marker	E-value
1	Syntrophomonas	rpo	-40
2	Syntrophothermus	ssu; rpo	-23; -20
3	Desulfatibacillum	ssu; rpo	-5; -19
4	Pelobacter	ssu	-65
5	Syntrophus	ssu; rpo	-9; -40.455
6	Syntrophobacter	ssu	-20

Table 2: Predicted presence of the syntrophic microbes based on protein homology with marker genes. The marker genes selected for obtaining the strains were Sigma Factor (rpo) and Small Subunit (ssu).

the sequence correspondence to Desulfatibacillum (average e value = -5 and -19), Syntrophus (average e value = -9 and -40.455) towards marker genes ssu and rpo respectively whereas Pelobacter (average e value = -65) for the rpo biomarker in the metagenome. To the best of our knowledge, this phenomenon has been revealed for the first time from the Jharia coal bed basin. There are reports related to the bacteria like *Desulfatibacillum alkenivorans* to be involved in syntrophic alkane metabolism in absence of sulphate [19]. Member of Syntrophaceae family *Syntrophus aciditrophicus* is linked with the degradation of monoaromatic compound like benzoate follows β-oxidation pathway for the production of acetate by syntrophic acetate oxidation [20]. Also, *Pelobacter carbinolicus* [21] from representing Pelobacter genus in the absence of sulphate have been found to be capable of utilizing organic acids and alcohols by forming a syntrophic union with a hydrogen-utilizing partner to reduce inhibition by hydrogen [22].

Conclusion

This is the first study from the Jharia basin depicting the presence of the syntrophic microbes mainly from Proteobacterial and Firmicutes phyla. The existence of syntrophic community in the subsurface microbiome indicates the possibility of existence of the substrates for hydrogenotrophic methanogenesis via volatile fatty acid degradation hence, making such pathways prevalent. Detailed presence of microbes in the microbiome is being carried to prepare a clear version of the methane formation from the initial point of the coal biotransformation which remains in its virginity due to the lack of proper data mining.

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