



Exploring the Role of Microbial Communities in the Formation and Fossilization of Coal Deposits from West Bengal Mines

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Abstract

This study investigates the crucial function of microbial populations in coal formation in West Bengal, India, specifically the Raniganj and Jharia coalfields. We used metagenomic analysis, isotopic research, and experimental simulations to investigate the role of ancient and present microbes to coalification. Key findings demonstrated that anaerobic bacteria such as *Clostridium spp.* and *Desulfovibrio spp.*, as well as methanogenic archaea, enhanced organic matter decomposition and mineralization. Isotopic studies revealed microbial activity in carbon cycling and sulfate reduction, with $\delta^{13}\text{C}$ depletion and $\delta^{34}\text{S}$ enrichment. Experimental simulations reproduced coal-like material production, underlining the importance of microorganisms in coal preservation. This study sheds light on microbial-geochemical interactions and proposes potential biotechnological applications in energy production and carbon sequestration.

Keywords: microbial, deposits, metagenomics, isotopic

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Introduction

Microorganisms are recognized to play important roles in the creation and preservation of coal and other fossilized materials (Akimbekov et al., 2022); (Ortiz-Castillo et al., 2021); (Dhami et al., 2023). However, there are significant gaps in our understanding of these processes, such as the exact mechanisms of microbial mineralization, the history of microbial communities in ancient environments, and the effect of environmental changes on microbial fossilization processes.

Addressing these gaps through interdisciplinary study is critical for advancing paleontology and better understanding the complex interactions between living forms and the Earth's geosphere across time.

Coal deposits, notably those in West Bengal, India, are a valuable geological resource created over millions of years from the remains of ancient forests and other organic matter (Saha et al., 2022); (Mondal et al., 2018). These deposits offer a unique opportunity to investigate the interactions of microbes and organic matter during the coalification process (Wang et al., 2018). The proposed study will investigate the microbial communities associated with these coal seams, both in ancient and modern contexts, to better understand their involvement in coal formation, fossilization, and the preservation of ancient biological components. The objectives of this project were: To characterize the microbial communities present in coal samples from West Bengal mines using metagenomic analysis; To investigate the isotopic signatures of microbial metabolisms in coal deposits and associated sediments; To experimentally simulate microbial processes involved in the coalification and fossilization of organic matter; To reconstruct the paleomicrobiomes of ancient coal-forming environments using molecular techniques.

Methodology

The methodology used to investigate microbial populations involved in coal production in West Bengal was systematic (Jha et al., 2022). To capture various microbial communities, samples were taken from the Raniganj and Jharia coalfields, which included both active and abandoned mines. Coal samples from various depths, including lignite and bituminous coal varieties, as well as surrounding sediment samples, were collected to better understand the ecological context of coal formation (Gopinathan et al., 2022); (Chawla et al., 2023). The samples were stored in sterile, airtight containers and transported at 4°C. Coal samples were coarsely ground, whereas sediment samples were homogenized for additional analysis (Matusiewicz, 2017). Microbial DNA was extracted using a procedure developed for low-biomass samples, which included bead-beating and enzymatic lysis (Pérez-Brocal et al., 2020). NanoDrop and Qubit were used to determine DNA quality, with degraded samples undergoing extra purification steps. Metagenomic sequencing was done using the Illumina NovaSeq platform, which used both shotgun metagenomics and 16S rRNA gene sequencing. MetaPhlAn3 was used to perform quality control, assembly, and taxonomic categorization on sequencing data, while HUMAnN3 was used for functional annotations. Phylogenetic relationships were investigated using MEGA software, with a focus on microorganisms engaged in carbon and sulfur cycle (Allioux et al., 2022).

Results

The metagenomic investigation of coal samples from the Raniganj and Jharia coalfields revealed a microbial community dominated by anaerobic bacteria, including *Clostridium spp.*, *Desulfovibrio spp.*, and *Methanobacterium spp.* *Clostridium species*, which are involved in organic matter fermentation, were numerous in all samples, indicating that they played a role in cellulose breakdown during early coal production. Sulfate-reducing bacteria (SRB), such as *Desulfovibrio spp.*, helped to generate pyrite (FeS₂), but methanogenic archaea, such as *Methanobacterium spp.*, produced methane, indicating active methanogenesis in deeper coal seams. Sediment samples had more microbial variety, with Proteobacteria, Firmicutes, and Cyanobacteria predominating. Cyanobacteria are believed to have contributed to primary production and nitrogen fixation, both of which are required for nutrient cycling during coalification. Sediments showed greater microbial diversity than coal seams, indicating more active environments. Table 1 shows a direct correlation between microbial composition

(*Clostridium spp.*, *Desulfovibrio spp.*, *Methanobacterium spp.*) and isotopic values ($\delta^{13}\text{C}$ and $\delta^{34}\text{S}$) in the metagenomic study. It also goes into detail on mineralization (Pyrite and Calcite), which were discussed earlier in the report.

Table 1. Microbial Abundance, Isotopic Ratios, and Mineral Composition in Coal Samples from Various Depths

Depth (m)	<i>Clostridium spp.</i> (%)	<i>Desulfovibrio spp.</i> (%)	<i>Methanobacterium spp.</i> (%)	$\delta^{13}\text{C}$ (‰)	$\delta^{34}\text{S}$ (‰)	Pyrite (%)	Calcite (%)	Quartz (%)	Other Minerals (%)
100	33.4	26.7	14.2	22.45	10.34	19.6	31.2	38.5	10.7
200	41.8	21.9	11.3	23.08	9.87	24.7	34.6	30.4	10.3
300	29.5	31.2	19.7	24.12	8.47	29.4	39.1	21.3	10.2
400	24.6	34.9	24.3	24.87	7.85	34.8	29.8	22.7	12.7
500	19.2	42.1	29.6	25.43	7.29	41.3	25.4	15.9	17.4

Isotopic analysis showed considerable $\delta^{13}\text{C}$ depletion in coal samples, indicating microbial breakdown by fermenters like *Clostridium spp.* Sulfur isotope analysis ($\delta^{34}\text{S}$) revealed enrichment, indicating active sulfate reduction by SRB. XRD and SEM examinations revealed the presence of pyrite and calcium carbonate (CaCO_3), indicating microbial-driven mineralization during coal production. Experimental simulations confirmed microbial roles in coalification, demonstrating a shift from fermentative bacteria to SRB and methanogens. Isotopic data from the simulations paralleled natural coal formation processes, whereas SEM imaging validated pyrite and calcite mineralization. Table 2 summarizes the KEGG pathway analysis discussed in the metagenomic functional annotation. It provides depth by explaining how the functional responsibilities of these pathways change with coal depth. Table 3 shows a clear comparison of microbial diversity, supporting the conclusions that sediment samples had more diverse microbial communities than coal samples, highlighting the contributions of Cyanobacteria and Proteobacteria.

Table 2. Functional Pathways (KEGG) vs Depth in Coal Samples

Depth (m)	Carbon Fixation Pathway (%)	Sulfate Reduction Pathway (%)	Methane Production Pathway (%)	Nitrogen Fixation Pathway (%)	Amino Acid Metabolism Pathway (%)	Others (%)
100	44.7	52.1	34.9	41.3	29.6	19.2
200	49.3	56.7	39.1	46.5	33.7	23.4
300	54.8	61.3	43.8	49.9	39.1	29

400	59.6	65.9	49.2	53.2	43.8	32.4
500	63.9	72.4	54.1	57.8	47.5	37.1

Table 3. Microbial Community Structure (Abundance %) in Sediments and Coal Samples from Raniganj and Jharia Coalfields

Sample Location	Depth (m)	<i>Clostridium</i> spp. (%)	<i>Desulfovibrio</i> spp. (%)	<i>Methanobacterium</i> spp. (%)	Proteobacteria (%)	Firmicutes (%)	Cyanobacteria (%)	Bacteroidetes (%)	Others (%)
Raniganj	100	31.2	24.5	16.7	11.4	9.8	4.7	3.1	2.3
Raniganj	200	36.4	21.1	12.3	14.7	10.1	2.8	4.5	2.5
Raniganj	300	27.6	33.5	21.9	19.8	5.9	3.5	1.9	5.9
Jharia	100	29.1	23.8	17.5	13.1	8.4	4.9	2.5	3.2
Jharia	200	33.7	20.5	16.1	15.3	7.7	3.1	4.1	3.3
Jharia	300	24.8	30.2	22.4	16.6	6.4	2.2	3.7	5.9

Ancient DNA (aDNA) study revealed ancient microbial taxa that are identical to present *Clostridium* and *Desulfovibrio* species, with evolutionary modifications in sulfur metabolism genes. Comparative study revealed that specialized ancient microorganisms developed into metabolically diverse modern groups. Data integration using PCA and NMDS revealed unique clusters of microbial communities based on depth and age, emphasizing the involvement of sulfur and carbon metabolism in coalification. Visualization methods, such as heatmaps and isotopic ratio plots, revealed a link between microbial activity and chemical changes in coal. 3D models of pyrite and calcite distribution provided more insight into the microbial-driven mineralization process. Table 4 supports the findings of genomic changes throughout time by emphasizing evolutionary differences in sulfur metabolism, methane generation, and organic matter degradation genes. Table 5 demonstrates quantitative connections between microbial abundance (*Clostridium* spp.), isotopic ratios ($\delta^{13}\text{C}$), and mineral composition (Pyrite), highlighting the importance of microbial activity in coal production.

Table 4. Comparative Genomics Analysis of Ancient and Modern Microbial Communities in Coal Samples

Genomic Feature	Ancient Microbes (%)	Modern Microbes (%)	Evolutionary Changes (Gene Conservation/Mutations)	Functional Impact on Coal Formation
Carbon Fixation Genes	58.3	71.5	Conserved	Improved carbon sequestration in modern communities
Sulfur Metabolism Genes	52.7	66.4	Minor Mutations	Enhanced sulfate reduction pathways

Methane Production Genes	49.9	68.2	Mutations in mcrA genes	More efficient methanogenesis Increased breakdown of complex organic matter Greater adaptation to extreme conditions in modern taxa
Organic Matter Degradation Genes	43.2	73.6	Major Gene Duplication Events	
Resistance to Environmental Stress	39.7	88.1	Extensive Evolutionary Divergence	

Table 5. Statistical Correlation of Microbial Abundance with Isotopic Ratios and Mineral Composition

Depth (m)	<i>Clostridium spp.</i> Abundance (%)	$\delta^{13}\text{C}$ (‰)	Pyrite (%)	Correlation (r) with $\delta^{13}\text{C}$	Correlation (r) with Pyrite (%)
100	33.4	-22.45	19.6	0.84	0.76
200	41.8	-23.08	24.7	0.81	0.74
300	29.5	-24.12	29.4	0.79	0.71
400	24.6	-24.87	34.8	0.77	0.69
500	19.2	-25.43	41.3	0.74	0.66

Discussion

(Sela-Adler et al., 2017), used IRMS to analyze $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ ratios to identify microbial metabolic activities, including methanogenesis and sulfate reduction. XRD was used to evaluate mineral composition, and SEM-EDS were used to investigate the microstructure and biomineralization of coal samples (Ali et al., 2023). Controlled microcosms were used to replicate ancient wetland conditions and monitor microbial activity, gas production, and organic matter decomposition (Romeijn et al., 2019). The development of coal-like compounds was tracked using SEM and XRD to determine mineralization. Ancient microbial communities were recreated using ancient DNA (aDNA) extracted from coal samples containing preserved plant material (Rivera-Perez et al., 2016). (Tawfik et al., 2018), sequenced aDNA using the Illumina MiSeq platform and compared the obtained profiles to modern microbial communities. Comparative genomics aids in the identification of conserved genes and evolutionary alterations. Functional pathways were recreated using KEGG and HUMAnN3, with an emphasis on their functions in coal production. Finally, associations between microbial communities, isotopic data, and coal properties were investigated using statistical methods such as PCA and NMDS, while heatmaps and scatter plots were used to illustrate microbial abundance and isotopic ratios (Paliy & Shankar, 2016); (Chen & Feng, 2023); (Monaco et al., 2020). This integrative strategy used microbial, chemical, and isotopic investigations to better understand the role of microbial communities in coal production.

Conclusion

This initiative emphasizes the importance of microbial communities in coal formation, specifically in the West Bengal coalfields. Anaerobic bacteria such as *Clostridium spp.*, *Desulfovibrio spp.*, and *Methanobacterium spp.* play critical roles in organic matter decomposition and pyrite and calcite mineralization, according to metagenomic, isotopic, and experimental

investigations. Isotopic signals ($\delta^{13}\text{C}$ depletion, $\delta^{34}\text{S}$ enrichment) indicate microbial influence on coal geochemistry. Experimental models effectively duplicated these processes, confirming microbial involvement. Paleomicrobiome reconstruction uncovers ancient microbial communities that resemble modern ones, providing insights into their evolutionary adaptations throughout coalification.

Conflicts of Interest

The authors declare no conflict of interest

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