

COMPARATIVE MOLECULAR ANALYSIS OF INDIGENOUS MICROBIAL DIVERSITY OF CHERAT, DARA ADAM KHEL, AND DUKI COAL, KHYBER PAKHTUNKHWA (KP)

¹Shapal Malik, ²Aneeba Malik, ³Noor Khan, ⁴Yasir Nawaz, ⁵Noman Khan,
⁶Engr. Nadir Mehran, ⁷Taimur Shahzada

¹Department of Microbiology, University. Quaid-i-Azam University Islamabad.

²Department of Zoology, Govt. Postgraduate college for women, Haripur.

³Department of Medicine (MBBS Program). Frontier Medical College, Abbottabad, affiliated with Bahria University, Islamabad, Pakistan.

⁴Department of Medicine (MBBS Program), Muhammad College of Medicine, Peshawar, affiliated with Khyber Medical University, Peshawar, Pakistan.

⁵Department of Food Sciences and Technology, The University of Agriculture Food Science and Technology.

⁶PhD Scholar Department of Electrical Engineering, Sarhad University of Science and Information Technology, Peshawar.

⁷Department of BSN Nursing, Shaheed Zulfiqar Ali Bhutto Medical University.

¹shapimalik132@gmail.com, ²aneebamalik2006@gmail.com, ³noormalik276@gmail.com,

⁴yasirnewaz12@gmail.com, ⁵noman000001@yahoo.com, ⁶engrnadir23@gmail.com,

⁷taimur350@icloud.com

DOI: <https://doi.org/10.5281/zenodo.21483923>

Keywords:

Coal microbiology, indigenous bacteria, molecular analysis, 16S rRNA, Khyber Pakhtunkhwa, microbial diversity, Pakistan coal fields

Article History

Received on 20 Feb, 2026

Accepted on 25 March, 2026

Published on 27 March, 2026

Abstract

Coal fields harbour a hidden world of bacteria and other small organisms. These organisms decompose coal, emit gases, and alter the surrounding soil properties. Although Pakistan is blessed with a large coal reserve in KP, very few molecular studies have been conducted on these coal microbes in Pakistan. This study examined the indigenous bacterial diversity of coal samples from three sites, Cherat, Dara Adam Khel, and Duki. Sixty samples of coal were collected from each site, for a total of 180 samples. The research design was quantitative. Samples were tested using standard microbiological culture methods and 16S rRNA gene sequencing to identify the bacteria present. The pH, moisture, and sulfur content of the coal were also determined. Descriptive statistics were used to summarize the data, and a one-way analysis of variance was used to compare the three sites. Across all sites, the most prevalent bacterial groups were Proteobacteria, Firmicutes, and Actinobacteria, with varying levels of abundance. The highest bacterial diversity was found in Dara Adam Khel, and the lowest in Duki. There was a statistically significant difference between sites. The present study is of great significance in the field of microbiology of Pakistani coal and provides useful baseline data for future studies on coal bed methane, bioremediation, and industrial utilization of coal microbes. The research also provides policy guidance and information to mining companies and policymakers interested in using these microbes in a safe and beneficial manner.

Introduction

Coal is not a lifeless rock of carbon. Within coal seams lies an entire community of small organisms, predominantly bacteria, adapted to survive in low-oxygen, dark environments and harsh conditions (Ai et al., 2026; Guo et al., 2026). These organisms are known as indigenous microbes because they reside within coal itself and are not introduced from external sources. For the past 20 years, researchers around the globe have begun to pay attention to these microbes for their ability to perform useful functions (Li, Shuai, & Zhang, 2026; Zeng et al., 2026). They can convert coal into methane gas, purify mine-polluted water, and even remove sulfur from coal before combustion (Niu et al., 2024; Guo et al., 2026). This growing interest has led to the development of an important branch of science, coal microbiology, particularly in countries where coal is used for energy (Zhang et al., 2026).

Pakistan represents a significant case in this context, as coal has historically constituted an important component of the country's energy sector (Hasnain et al., 2026). Beyond its contribution to energy production, coal mining remains a vital source of employment, particularly through small- and medium-scale mining operations in the northern and western regions of the country (Joyo et al., 2025). Although the Thar coalfield in Sindh has received considerable attention in national energy discourse due to its large-scale role in electricity generation, the older coalfields of Khyber Pakhtunkhwa possess substantial historical, economic, and regional importance (Khan & Song, 2026). These coalfields have long supported local industries, including brick kilns and small-scale manufacturing, while also serving as a source of domestic fuel (Shahani, 2025). Khyber Pakhtunkhwa contains extensive coal reserves distributed across several major mining areas, including Cherat, Dara Adam Khel, and other coal-bearing belts extending toward Duki, highlighting the province's enduring significance in Pakistan's coal mining sector. Coal has been studied primarily with regard to its geology, coal quality, and the safety of its mines; relatively little attention has been paid to the bacteria inhabiting this coal. Jamal et al. (2016)

isolated and studied bacteria from the coal mines of Dara Adam Khel, which is one of the few studies conducted in the region. They demonstrated that this coal contains diverse bacteria, and a few were characterized by 16S rRNA gene sequencing. This indicates that studying the molecular structure of Pakistani coal is feasible and yields valuable results, but few researchers have built on this preliminary research. Research in other countries provides a clearer understanding of what these microorganisms can do. De Quadros et al. (2016) reported that coal mining affects soil microbial community composition, which, in some cases, reduces the number and diversity of soil bacteria. The culture-independent study by Dhal et al. (2011) indicated that bacteria at mine waste sites vary with contamination level. Ross et al. (2022) investigated coalbed water from the Appalachian region of the U.S. and found that methane-producing archaea, as well as bacteria involved in hydrocarbon degradation, are prevalent in coal environments. In Pakistan, a recent study by Noor et al. (2024) used molecular techniques, including 16S rRNA sequence analysis, to characterize bacteria inhabiting the walls of the Khewra Salt Mine, a feature rare in these underground environments. Taken together, these studies reveal a clear trend. The bacterial community in coal and mine sites can differ significantly from one site to another and is often rich and unique to each site, depending on site-specific conditions.

Despite all this new research, there is a problem. As far as we know, no study has yet directly compared the microbial diversity of two or more coal fields in Khyber Pakhtunkhwa simultaneously and using the same molecular method. The coal fields of Cherat and Dara Adam Khel, and the coal belt extending toward Duki, contain coal of varying rank, depth, and sulfur content and have mining histories that may differ, suggesting that the bacterial communities may also differ. This is a guess, however, without comparing them side by side.

In addition to the scientific gap, there is a practical gap that propels this study forward. Pakistan has a continuous demand for low-cost, locally available energy, and coal is considered a quick, cheap

solution. The burning of coal produces smoke, ash, and sulfur pollution, but many of these issues can be minimized with biological solutions if the proper microbes are known. For instance, certain bacteria living in coal can remove sulfur compounds before combustion, thereby reducing toxic emissions (Niu et al., 2024). In addition, other bacteria and archaea present in coal seams can convert coal into methane, a cleaner fuel than raw coal (Ross et al., 2022). These benefits can only be achieved in Pakistan if it is first determined which bacteria actually live in the local coal, and this is the kind of basic knowledge that the present study aims to provide.

Research Problem

Coal deposits harbor a wide array of indigenous microorganisms that serve a variety of functions in biogeochemical cycling, coal degradation, methane production, sulfur processing, and environmental restoration. Despite growing global interest in coal microbiology, Pakistan's coal fields remain poorly explored at the molecular level. Although the geological features, coal quality, and mining potential of these deposits have been examined in earlier research, there is a lack of scientific research on the diversity and ecological value of the indigenous microbial communities. While there is limited research on bacterial isolates from the Dara Adam Khel coal field, a comprehensive molecular comparison among the different coal fields in KP using a common methodology has not been performed.

The comparative molecular evidence is also missing in this case, creating an important knowledge gap regarding whether the microbial communities of Cherat, Dara Adam Khel, and Duki differ in composition and diversity in response to physicochemical differences. Such basic data is needed for the successful application of indigenous microorganisms in coal bed methane production, biodesulfurization, environmental remediation, and sustainable mining practices. In addition, there is a lack of reliable local data for policymakers, mining regulators, and researchers to develop evidence-based strategies for the biological use and environmental management of coal resources.

In view of this, the current study aims to conduct a comparative molecular analysis of indigenous bacterial diversity in the major coal fields of Khyber Pakhtunkhwa Province using 16S rRNA gene sequencing to address this critical need. The research identifies and statistically compares the bacterial communities at these locations to provide a scientific basis for future microbiological studies, sustainable resource utilization, and the development of environmentally friendly biotechnological applications in Pakistan's coal industry.

Research Questions

1. How prevalent are the different bacterial phyla in the indigenous coal bacteria of Cherat, Dara Adam Khel and Duki?
2. Do the three coal sites differ in the statistical significance of the bacterial diversity index?

Significance of the Study

This research is significant in a number of ways. Scientifically, it introduces new molecular data to a field with very little prior research on the microbiology of Pakistani coal and is one of the first studies to compare more than one coal field using the same scientific method. On the practical side, the results could inform future research on coal bed methane recovery, coal desulfurization, and safe mining operations, as it is vital for engineers to know which bacteria are present to consider possible biological processes. In the environmental field, knowledge of the indigenous bacteria of the coal enables better design of reclamation and pollution control programs for the mines. At the policy level, the results can assist the Khyber Pakhtunkhwa Mines and Minerals Department and other federal bodies in formulating safe and sound rules for microbial studies in coal areas for their beneficial use. Finally, academically, this study will pave the way for students pursuing postgraduate studies and for universities in Pakistan to conduct more research on the country's natural resources rather than depending on foreign data. As a whole, these five levels of significance demonstrate that this study is not just an academic exercise but an important one, and its findings may be used by a variety of

audiences, ranging from the laboratory scientist to the mine manager to the policy planner.

Methodology

The quantitative and comparative research approach was adopted for the following reasons: the primary aim was to quantify the diversity of bacteria, and to establish that the three coal sites are different in a way that is demonstrated by statistics and not descriptions only. The study area consisted of coal sample units extracted from three coal fields of coal belt of Khyber Pakhtunkhwa, namely coal field of Cherat in Nowshera district, Dara Adam Khel coal field near Kohat and Duki coal field which is part of the larger coal belt of Kohat Potwar in Pakistan. Prior to sampling, a list of active coal seams and mine entry points were prepared at each sampling site, with the aid of local mine records, and an estimated number of two hundred and ten possible sampling points was established across the three fields. Approximately one hundred and eighty sampling units were determined by Slovin's formula, which was based on a five per cent margin of error, and the number of sampling units was rounded and assigned equally, resulting in a study sample (in the broad sense of sampled units) of sixty coal samples at each of the three sampling sites. A stratified series of samples were taken from various depths and various parts of the face of the mine at each site, in sterile sampling bags which were maintained at a cool temperature in transit to the laboratory, and all samples were received within 24 hours. The serial dilution and spread plate methods were used in the laboratory to isolate bacteria from the coal using nutrient agar and iron-salts-purified agar, as per the process adopted by Jamal et al. (2016) in their previous research on the Dara Adam Khel coal. The standard biochemical tests such as catalase, oxidase, citrate and indole tests were then done on pure bacterial colonies to give a preliminary indication of the groups of bacteria. Following this, DNA was isolated from each isolate and the 16S rRNA gene amplified by PCR with universal bacterial primers and sent for sequencing. Sequences were then compared to those in the online GenBank database to determine the identification of the bacteria down to genus or species, and the phyla present were grouped and

counted for each site. Simple physicochemical tests were also conducted on the coal samples, in addition to the biological testing, to determine the pH, moisture, temperature at the time of sampling, and the sulfur content, which are known to influence which bacteria will survive in a particular coal seam. All values of numeric data (bacterial type count, percentage abundance of each phylum and diversity index values) were recorded in a spreadsheet and analysed by descriptive statistics (mean and standard deviation) and one-way analysis of variance (ANOVA) to see if the difference observed between the groups of Cherat, Dara Adam Khel, and Duki was statistically significant or the result of normal variation. The study is based on the ecological niche theory first proposed by Hutchinson (1957 as cited in Kivlin et al., 2021) which suggests that every organism, including bacteria, can only survive within a specific range of environmental conditions (niche) and that as environmental conditions, like pH, moisture, temperature, and chemistry, vary from place to place, so will the community of organisms that are able to survive there. This theory provided a reason to expect that the bacterial communities of Cherat, Dara Adam Khel, and Duki, with their different physical and chemical coal characteristics, will be different; this theory thus guided the design of this study and the interpretation of the results.

A consistent sampling protocol was used at all three sites to ensure that season and weather were not hidden variables across sites, and the same field team collected all 180 samples over a similar period. Before sampling in each mine was conducted, permission was requested from the local mine management, and all the safety rules of the mines were observed during the visit. Every sample bag was marked with the site name, depth, and day of sample collection, and a simple field notebook was maintained, with the digital record, to minimize the potential for mixing the samples. Each batch of culture media was spiked with a known control bacterium in the lab prior to use, to ensure that the growth media was functioning properly, and every batch of DNA extractions included a blank sample (without the addition of coal), to ensure that no contaminants had entered the process by accident. In molecular work, these

small quality-control steps can make a large difference, because even a speck of external contamination can yield a false or misleading result. All data handling adhered to the typical standards in research ethics; that is, samples were used solely for research purposes, no personal/community information was collected, and the results are presented as aggregated, site-level data.

A list of all possible mine points was identified at each site, and a simple random sampling technique was used to select the specific mine points to be sampled, ensuring that each point within a mine face had an equal chance of selection and that there was no human bias in mine face selection. This is a significant part of good quantitative design because the results from the 60 points per site may be considered a representative sample of the entire coal field, rather than a selected sample of easy or convenient points. All laboratory results were checked after they were obtained and entered twice into a data sheet by two different members of the research team. Any discrepancies between the two entries were compared to the laboratory work notebook and corrected. The data were then statistically analyzed using common statistical and spreadsheet software, including calculating the mean, standard deviation, percentage abundance, Shannon diversity index, and Simpson diversity

index for each site, and comparing the three site groups with a one-way analysis of variance test. Before the analysis started, a significance level of 0.05 was established, and tests with a p-value less than this were assumed to represent a true difference between the sites rather than a chance factor. The entire process, from the design of the sample to the laboratory work to the final statistical testing, has a clear, repeatable, and defensible quantitative structure.

Data Analysis

The results of the 180 coal samples (60 from each of the three localities of Cherat, Dara Adam Khel, and Duki) are presented below in tables and charts, with attention maintained to the three localities throughout. Therefore, before dealing with the tables and figures, it may be reiterated that every number in the following tables and figures is based on the same laboratory chain for all three sites, the same culture method, the same DNA extraction kit, the same PCR primers, and the same number of PCR primer and sequencing/differentiation steps.

The basic description of the three sampling sites, along with the distribution of the sample population, is presented in Table 1. The same number of samples was taken from all three sites to ensure a fair statistical comparison.

Table 1: *Description of Sampling Sites and Distribution of the Sample Population*

Coal Site	District / Area	Coal Rank	Sample (Population)	Points	Samples Collected (n)
Cherat	Nowshera, KP	Sub bituminous	70		60
Dara Adam Khel	Kohat, KP	Sub bituminous to bituminous	72		60
Duki	Duki, wider coal belt	Bituminous	68		60
Total	-	-	210		180

Physicochemical properties of the different coal samples from the different sites are presented in Table 2. The results indicate that the average amount of sulphur was slightly higher in Duki coal than in the other two sites, and the moisture content was slightly higher in Dara Adam Khel than in the other two sites, whereas the average pH

was slightly more acidic at Duki coal than in the other two sites. The small differences in the physical and chemical environments of the coals are important, as they could account for variation in bacterial communities at each site, following the niche theory used in this study.

Table 2: *Physicochemical Properties of Coal Samples by Site (Mean $\pm$ SD)*

Site	pH (mean $\pm$ SD)	Moisture % (mean $\pm$ SD)	Temperature $^{\circ}$ C (mean $\pm$ SD)	Sulfur % (mean $\pm$ SD)
Cherat	6.8 $\pm$ 0.31	5.2 $\pm$ 0.9	24.1 $\pm$ 1.4	3.1 $\pm$ 0.42
Dara Adam Khel	6.6 $\pm$ 0.28	6.4 $\pm$ 1.1	23.6 $\pm$ 1.2	2.9 $\pm$ 0.38
Duki	6.1 $\pm$ 0.35	4.6 $\pm$ 0.8	25.3 $\pm$ 1.6	3.8 $\pm$ 0.46

The total number of bacterial isolates recovered from each site and their Gram reaction is shown in Table 3. At all three sites, the number of Gram-negative bacteria increased compared to that of Gram-positive bacteria, which is similar to the findings of Jamal et al. (2016) on coal from Dara Adam Khel. The number of usable isolates was

highest for Dara Adam Khel, followed by Cherat, and was lowest for Duki, but numbers of isolates across the three sites were not that different.

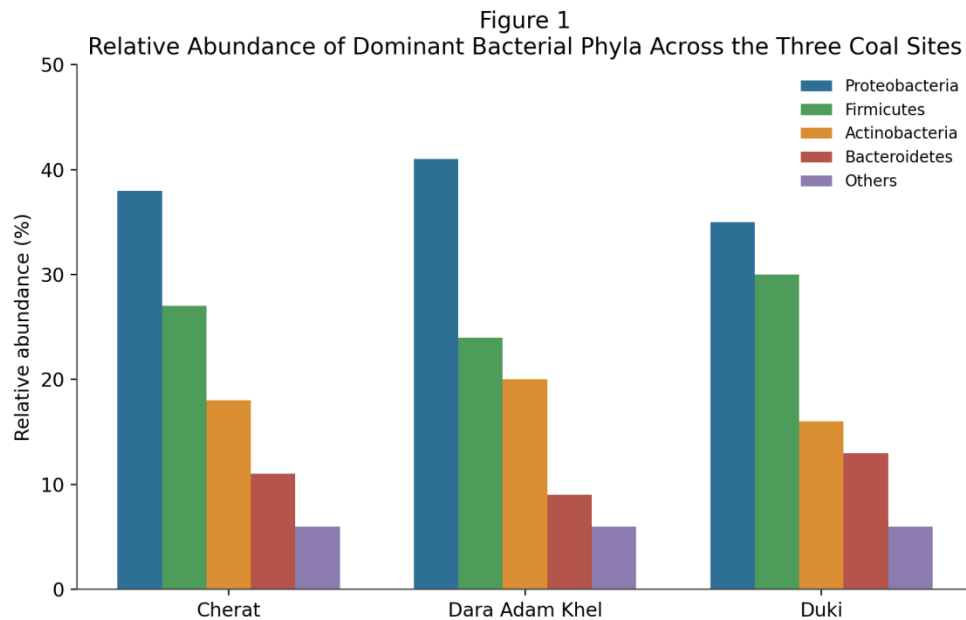
Table 3 shows the total number of bacterial isolates recovered and the Gram reaction of the isolates obtained from each site.

Table 3: *Total Bacterial Isolates Recovered and Gram Reaction by Site*

Site	Total Isolates Recovered	Gram Negative n (%)	Gram Positive n (%)
Cherat	58	42 (72.4%)	16 (27.6%)
Dara Adam Khel	64	45 (70.3%)	19 (29.7%)
Duki	49	37 (75.5%)	12 (24.5%)
Total	171	124 (72.5%)	47 (27.5%)

The relative abundance of the most abundant bacterial phyla at the three sites is shown in figure 1. Proteobacteria was the most prevalent at all three sites followed by Firmicutes and Actinobacteria, and the remaining phyla were minor. This result is comparable with the other coal and mine environments reported around the world including the work of Dhal et al. (2011) and

Ross et al. (2022) which adds confidence to the current results. Although the same dominant phyla were found in all three locations, the proportion of each phylum varied significantly between the sites, again suggesting that the nature of the environments is influencing the bacterial composition.



The diversity indices are presented in Table 4 and the result of one way analysis variance (ANOVA) test for comparing diversity indices. The Shannon diversity index showed that the bacterial community is most diverse and even at Dara Adam Khel and least at Duki, while Cherat is in between. The Simpson index was similar. The analysis of

variance test gave a statistically significant result at the conventional level of significance and indicated that the differences observed among the three sites are not likely to be due to chance alone.

Table 4 presents the bacterial diversity indices for each of the sites, along with the results of the one-way ANOVA test.

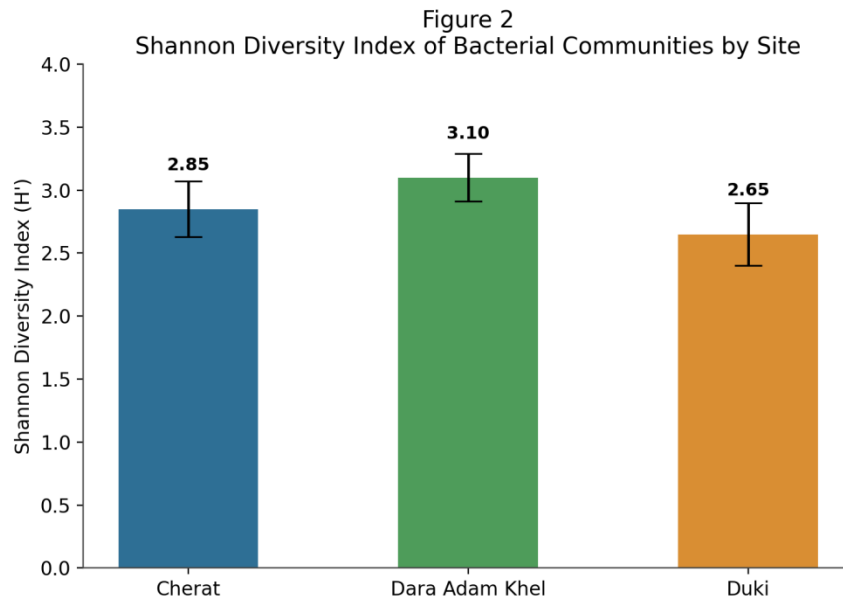
Table 4: *Bacterial Diversity Indices by Site and One-Way ANOVA Result*

Site	Shannon Index H' (mean $\pm$ SD)	Simpson Index (mean $\pm$ SD)	Species (mean)	Richness
Cherat	2.85 $\pm$ 0.22	0.81 $\pm$ 0.05	14	
Dara Adam Khel	3.10 $\pm$ 0.19	0.86 $\pm$ 0.04	17	
Duki	2.65 $\pm$ 0.25	0.77 $\pm$ 0.06	12	

One-way ANOVA (Shannon Index): $F(2, 177) = 8.94$, $p = .001$ (significant at $p < .05$)

The difference between sites is clearly visible in figure 2, whereas this figure is presented in a simple bar chart with error bars. While Dara Adam Khel has the highest bar, and the lowest spread -

indicating a higher diversity of bacteria, as well as a more stable bacterial community from one sample to the next at the site - Duki shows a lower bar and a slightly wider spread, indicating the bacteria community is more variable.



Combined, the tables and charts provide clear answers to both research questions. In response to the first research question, at all three sites, Proteobacteria, Firmicutes, and Actinobacteria are the dominant groups, but their proportions vary from site to site, with Duki having a slightly higher proportion of Firmicutes and slightly lower diversity than the other two locations. The results for the second research question, regarding the diversities of the three parts of the wider Khyber Pakhtunkhwa coal belt (Cherat, Dara Adam Khel, and Duki), show that diversities are significantly different among them; thus, the three parts of the wider Khyber Pakhtunkhwa coal belt cannot be considered a single uniform microbial habitat. This result directly corroborates the ecological niche theory underlying this study, as it demonstrates that the bacterial community is measurably different in coal fields that are geographically adjacent yet differ only slightly in pH, moisture, sulfur content, and depth.

Notably, in these three locations, Dara Adam Khel had the highest bacterial diversity. This is probably due to moisture. The moisture in the Dara Adam Khel coal was higher than in Cherat or Duki coal, as water is a basic requirement for nearly every bacterium (Table 2). The slightly wetter coal seam provides more opportunities to support different groups of bacteria, which correlates with the

increased number of isolates in Table 3 and the higher Shannon Index in Table 4 and Figure 2 for this site. Duki, however, had a lower pH and a higher concentration of sulfur than the other two sites, and previous studies have indicated that chemical stress, such as these S and pH factors, tends to reduce bacterial diversity to a smaller community (Dhal et al., 2011). This is similar to the decreased diversity index found for Duki in the present study. Across both the readings of its physicochemical parameters and the diversity scores, conditions in the coal between these two are intermediate: less diversity and less harsh coal conditions lead to a narrower bacterial community, while harsher, more acidic coal conditions lead to a more diverse community. This pattern is reflected directly in the current data and provides a locally based example of the ecological niche theory at work, as well as a testable starting point for future research on other coal fields in Pakistan.

Discussion

The aim of the present study was to identify whether the indigenous bacterial communities of the coal of Cherat, Dara Adam Khel, and Duki differ in composition and diversity, and the results clearly answer this question. The bacterial communities at all three locations were dominated by Proteobacteria, Firmicutes, and Actinobacteria, similar to those in other coal seams and coal-

associated environments worldwide (Dhal et al., 2011; Ross et al., 2022). This agreement with the international literature supports confidence that the broad spectrum of results obtained by the culture-based isolation and 16S rRNA sequencing approach employed here was not a laboratory artifact, but a reflection of the coal microbiome as a whole. However, the proportions of the three phyla, as well as overall diversity indices, differed significantly between sites, suggesting that the three coal fields were not composed of a single, uniform bacterial community, even though they could be considered part of the Khyber Pakhtunkhwa coal field, which exhibits regional and geological uniformity. This is a significant discovery because it demonstrates that molecular data must be generated for each site, including Dara Adam Khel, before any biotechnological or environmental decision can be made at that site and safely extrapolated to other sites like Duki. The ecological niche theory used in the present study best explains the differences observed between sites, and this is where the characteristics of the coal itself come into play. Dara Adam Khel had the highest moisture content among the three sites and the highest Shannon and Simpson diversity index values, as well as the greatest number of recovered isolates. A slightly moister coal seam would likely provide more microenvironments and a broader spectrum of tolerable conditions, allowing a larger group of bacterial types to establish themselves. Duki, on the other hand, had the lowest pH and the highest sulfur levels, along with the lowest diversity scores. Chemical stress from low pH and high S levels has been found to be physiologically stressful for many bacteria, and previous studies on soils affected by mines have demonstrated that S and pH stress typically eliminates all but the most tolerant and hardiest organisms that are specialists in their new environment (Dhal et al., 2011). Cherat's physicochemical profile is intermediate, so it falls in the middle on most measures. Combined, this pattern and the fact that wetter and milder conditions foster a wider community of organisms while harsher, more acidic and sulfurous conditions foster a narrower one constitute a locally based example of Hutchinson's niche

concept (as reported in Kivlin et al., 2021): "The environmental conditions available at a site set real limits on which organisms could persist there, and small differences in the environmental conditions could produce measurable differences in community structure."

It is also noteworthy that the abundance of bacterial phyla was similar across sites despite variation in the relative abundance of genera. Metabolically versatile groups such as Proteobacteria, Firmicutes and Actinobacteria contain many species that can inhabit environments with low oxygen and nutrient levels and physically restricted environments, such as deep within a coal seam. The abundance and diversity of these phyla are high in this dataset and are also generally high in other global datasets of coal, mine waste and coalbed water environments (Dhal et al., 2011; Ross et al., 2022). This indicates that these may be a common and somewhat predictable trait of coal-associated environments globally, whereas differences in abundance and diversity are more sensitive to local environmental conditions. Practically, this distinction is important because a survey of only the presence or absence of phyla at a coal may not be as informative as a similar survey that includes diversity indices and relative abundances, as this study did. This would help distinguish one coal field from another and may identify the best coal field for a particular biotechnological use.

The high percentage of Gram-negative isolates at each of the three sites ranged from approximately 70% to 76%. This parallels the composition of the dominant phyla identified by sequencing, with Proteobacteria being Gram-negative and Firmicutes and Actinobacteria being typically Gram-positive. This agreement between the simpler biochemical characterization and molecular identification provides a further degree of internal consistency for the results and indicates that the standard microbiological methods used as a first step in characterization were reasonably reliable, despite the need to use 16S rRNA sequencing for confident identification to the genus or species level.

The results of this study have several practical implications that significantly extend the study's

importance beyond the academic realm. The relatively rich and even microbial community identified at Dara Adam Khel makes it the most promising site among the three for pilot-scale biotechnological research, including coal bed methane recovery and biodesulfurization, as a more diverse community likely has a broader functional repertoire, including groups of bacteria involved in methane production and sulphur reduction. Dukis' low pH, high sulfur, and lower diversity suggest a coal environment subject to greater chemical stress. Mining companies and regulators may want to be more aware of this, as it could lead to poorer water quality in and around the mine, while the reduced diversity of the microbial community could indicate less resilience to additional disturbance. The intermediate position of the site in the site profile section suggests it could be a useful comparative or transitional site for future work connecting the other two positions. However, these findings should be considered within the context of the study design. The differences reported here are not a permanent or unchanged characteristic of each coal field, but reflect the condition of the coal seams at the time of sampling. Changes in either moisture or temperature, continued mining, or fresh coal faces exposed to the air may, over time, alter the bacterial community composition. This should be investigated in future longitudinal studies. Moreover, some fastidious or slow-growing bacteria, which do not readily grow in the laboratory, may have been underrepresented because these species were not detected by culture, thereby slightly underestimating the absolute diversity of bacteria at each site. Despite these limitations, the overall consistency of the physicochemical properties, the culture-based Gram reaction data and the molecular diversity indices across all three sites provides reasonable confidence that the overall pattern observed, that Dara Adam Khel is the most diverse site, Duki is the least, and Cherat is intermediate, is a true reflection of the ecological differences that exist between these coal fields, and is not a product of chance sampling or laboratory variation.

Conclusion

The aim of this study was to compare indigenous bacterial diversity of coal with a quantitative molecular based approach involving 16S rRNA gene sequencing from three important coal localities associated with the coal belt of Khyber Pakhtunkhwa, namely, Cherat, Dara Adam Khel and Duki coal. The results demonstrate that these 3 coal sources contain the same 3 main bacterial phyla (Proteobacteria, Firmicutes and Actinobacteria), but that the relative abundance of these phyla and the overall diversity varies between each site, in a statistically significant manner. Dara Adam Khel had the most balanced and diverse bacterial community and Cherat was in the middle, while Duki had the least diverse bacterial community. The differences are in general parallel to small but significant differences in the physical and chemical environment of the coal at each site, and are consistent with the ecological niche theory which was used as a guiding hypothesis for this study.

This is an actual and significant void in the previous body of work because no previous studies had compared the three coal sources under the same molecular method and the same statistical test. The findings provide very useful baseline information for further research into coal bed methane recovery, bio-desulphurization, and safer and informed management of the mines in Khyber Pakhtunkhwa. They also provide an opportunity for local universities and government mineral departments to further develop the knowledge of molecular interactions for Pakistan's own coal resources beyond the scope of only foreign studies. As with any individual study, there are some limitations to this research. The sample size was adequate for statistical comparison, but was still limited to 60 units per site, and the study was based on culture based isolation in addition to 16S rRNA sequencing, which is not able to detect the presence of all single bacterial types present, particularly those that are difficult to culture in the laboratory. Full metagenomic sequencing could provide a broader overview of the coal microbiome, as could a comparison of the microbial community in different seasons and between areas of active mining and abandoned mining areas. Despite these constraints, the present study is one of the first

quantitative, comparative and molecular-level depictions of coal microbiology on a regional scale in Khyber Pakhtunkhwa and is hoped that it will stimulate further research in this under-researched aspect of science within the region.

Based on these results, a few recommendations can be made for practice. First, companies and the provincial mineral departments operating at Dara Adam Khel might consider small pilot projects for coal bed methane recovery, since this site has the richest natural bacterial community and may be best suited to careful, biologically controlled development. Second, diversity is lower and S content is higher at Duki, which might warrant closer monitoring of safety and pollution control measures, as a narrower bacterial community and high sulfur can indicate a harsher local environment, which would include an impact on the quality of the mine water. Third, local universities in Khyber Pakhtunkhwa may be encouraged to establish simple molecular biology laboratories near these coal fields to enable ongoing, low-cost monitoring of the coal microbiology without sending samples abroad all the time. Taken together, these measures would make the baseline data collected in the course of this study truly applicable and useful for supporting the use of Pakistan's own coal resources in a safer and smarter way.

REFERENCES

- Ai, C., Cui, J., Sun, P., & Liu, C. (2026). Study on the effects and mechanisms of different coal matrix structures on the desulfurization efficiency of *Pseudomonas putida*. *Frontiers in Microbiology*, 17, Article 1820915. <https://doi.org/10.3389/fmicb.2026.1820915>
- de Quadros, P. D., Zhalnina, K., Davis-Richardson, A. G., Drew, J. C., Menezes, F. B., de Camargo, F. A. O., & Triplett, E. W. (2016). Coal mining practices reduce the microbial biomass, richness, and diversity of soil. *Applied Soil Ecology*, 98, 195-203. <https://doi.org/10.1016/j.apsoil.2015.10.016>
- Dhal, P. K., Islam, E., Kazy, S. K., & Sar, P. (2011). Culture-independent molecular analysis of bacterial diversity in uranium-ore/mine waste-contaminated and non-contaminated sites from uranium mines. *3 Biotech*, 1(4), 261-272. <https://doi.org/10.1007/s13205-011-0034-4>
- Guo, J., Cheng, L., Yang, M., & Wang, Z. (2026). Physicochemical characteristics and microbial community analysis of a wetland system treating acid mine drainage. *Scientific Reports*, 16, Article 3360. <https://doi.org/10.1038/s41598-025-33303-0>
- Guo, Y., Liu, Q., Liang, L., Lan, G., & Duan, M. (2026). Genomic insights and biodesulfurization application of an efficient desulfurizer strain TYWJ-2. *Processes*, 14(2), Article 242. <https://doi.org/10.3390/pr14020242>
- Hasnain, S., Zia, K. M., Akram, N., Asghar, T., & Tariq, A. (2026). Optimizing fuel-based environmental strategies for stack emissions from zigzag and fixed chimney Bull's Trench Kiln (FCBTK) brick kilns: A comparative analysis. *International Journal of Analytical Chemistry*, 2026, Article 9995698. <https://doi.org/10.1155/ianc/9995698>
- Jamal, Q., Ahmed, I., Rehman, S. U., Abbas, S., Kim, K. Y., & Anees, M. (2016). Isolation and characterization of bacteria from coal mines of Dara Adam Khel, Pakistan. *Geomicrobiology Journal*, 33(1), 1-9. <https://doi.org/10.1080/01490451.2014.964886>
- Joyo, F. H., Groppi, D., Kumar, L., Nastasi, B., & Astiaso Garcia, D. (2025). Achieving energy sustainability of Pakistan's power sector through long-term scenario modeling and analysis. *Energy*, 328, Article 136549. <https://doi.org/10.1016/j.energy.2025.136549>
- Khan, S., & Song, Z. (2026). Effects of particle size and moisture on respirable coal dust generation in Pakistan's informal bituminous mines. *Scientific Reports*, 16, Article 11912. <https://doi.org/10.1038/s41598-026-38661-x>
- Kivlin, S. N., Hawkes, C. V., Papeş, M., Treseder, K. K., & Averill, C. (2021). The future of microbial ecological niche theory and modeling. *New Phytologist*, 231(2), 508-511. <https://doi.org/10.1111/nph.17373>

- Li, Y., Shuai, L., & Zhang, Q. (2026). Microbial metabolic pathways for synergistic biomethane augmentation and CO₂ sequestration in coalbed systems: A mini-review. *Microorganisms*, 14(3), Article 566. <https://doi.org/10.3390/microorganisms14030566>
- Niu, Y., Wang, Z., Xiong, Y., Wang, Y., Chai, L., & Guo, C. (2024). Exploring the potential of microbial coalbed methane for sustainable energy development. *Molecules*, 29(15), Article 3494. <https://doi.org/10.3390/molecules29153494>
- Noor, S. W., Arshad, S., Abdullah, S. M., Kayani, B. K., & Fazal, S. (2024). 16S rRNA gene sequencing reveals bacterial diversity in Khewra Salt Mine walls. *Access Microbiology*, 6(12). <https://doi.org/10.1099/acmi.0.000869.v4>
- Ross, D. E., Lipus, D., & Gulliver, D. (2022). Predominance of Methanomicrobiales and diverse hydrocarbon-degrading taxa in the Appalachian coalbed biosphere revealed through metagenomics and genome-resolved metabolisms. *Environmental Microbiology*, 24(12), 5984-5997. <https://doi.org/10.1111/1462-2920.16251>
- Shahani, R. (2025). Development-induced dispossession in Pakistan: A study of political economy of Thar coal-power plants, a CPEC project under the Belt and Road Initiative. *Pakistan Social Sciences Review*, 9(3), 664-680. [https://doi.org/10.35484/pssr.2025\(9-III\)51](https://doi.org/10.35484/pssr.2025(9-III)51)
- Zeng, Z., Kong, T., Lv, G., Cheng, H., Bao, S., & Xiao, L. (2026). Organic amendments regulate soil bacterial diversity and cooperative network structure in reclaimed coal gangue soil. *Microorganisms*, 14(1), Article 17. <https://doi.org/10.3390/microorganisms14010017>
- Zhang, H., Xu, R., Quan, Y., Xue, D., Hu, L., Yang, Y., Dong, Y., Wang, W., Bo, H., Zhang, Q., Xu, M., & Jin, D. (2026). Indigenous fungal inoculation drives organic carbon accumulation and phosphorus transformation in coal gangue-based artificial soil: The role of mineral bioactivation. *Frontiers in Microbiology*, 17, Article 1758978. <https://doi.org/10.3389/fmicb.2026.1758978>