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Cultivable microbial diversity study of AMD water samples from Rajpardi lignite mine, India

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ABSTRACT
The oxidative dissolution of metal sulphides generates acid mine drainage (AMD), which is characterized by strong acidity and high metal concentrations. Acidophiles are responsible to speed up the process of the generation of acid. Extreme environments are home to a variety of microorganisms that survive and thrive. Despite these, we know little about their large-scale ecological distribution patterns and controls. Microbial diversity analysis reveals the existence of diverse, yet largely consistent communities. Here, we assessed the Rajpardi mine bacterial community composition and diversity using a cultivable approach. A total of 187 heterotrophic isolates were successfully cultivated from the collected water and sediment samples of the Rajpardi Lignite mine, in Gujarat, India. The isolates were characterized based on cell and colony morphology. The highest microbial growth was obtained from sediment samples on the YEA medium. The highest relative density and frequency of isolates 2 and 28 indicate their presence in many samples. The sediment sample had the highest number of varieties and, thus, had the highest Dice matrix index value of 3.197, indicating a community with many taxa. Sample SS3 and WS4 showed the maximum Shannon index indicating a high similarity. This research also explored the relationship between the variance in heterotrophic microbial diversity of the studied lignite mine of Gujarat.
Keywords*:* Acid mine drainage, Cultivable approach, Microbial diversity, PCA analysis, Lignite mine, Diversity indices

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INTRODUCTION

With the enormous potential for worldwide expansion, microbes demonstrate nonrandom distribution patterns across a variety of habitats at different spatial scales, making microbial ecology a captivating field of study [1,2]. The harsh environmental conditions on the earth are home to a wide variety of unique microorganisms. Besides increasing knowledge about the ecology and evolution of microbes, the dynamics of these microbial communities and their interactions with physicochemical and geochemical factors will make it possible to reveal these microbial adaptations and tolerance power in different kinds of extreme environments. Using niche models that take environmental and geochemical data into account, the composition and diversity of microbial communities can be predicted. Due to environmental variety, dispersion limitations, historical contingency, stochastic events, and disruptions, microbial communities can also be affected by spatial distance and temporal distance [3,4]. Due to their extreme environments and abundance of energy-deriving processes, such as acidic systems, extreme environments might be helpful as model systems for the investigation of microbial niches [4]. Acid mine drainage (AMD), a common and frequent environmental issue associated with the mining of lignite at lignite mines, is caused by the oxidative dissolution of pyrite (FeS_2) and other sulfide minerals in an environment consisting of oxygen and water. Despite the presence of low levels of metabolically active microbial diversity found in these unique environments, such as well-adapted acidophilic microorganisms to a wide range of environmental

stresses and are primarily responsible for the generation of sulfuric acid and toxic metals-rich solutions [5,6]. More recently used molecular-based investigations have revealed highly reported acidophiles isolated from mining ecosystems. *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans, Ferroplasma* spp. and *Leptospirillum* sp. are widely implicated to be the microorganisms that have important roles in the pyrite dissolution *in situ* that control the rate of AMD generation [7]. In this context, a comprehensive understanding of the pattern of AMD microbial diversity, how geochemical factors influence the composition of microbial communities and whether these environmental key factors differ from those that affect the 'normal' environment has not yet been clear [8]. The culturing techniques provide knowledge of characterizing cultivable microbial communities in large numbers of ecological samples to examine broad trends of microbial distribution in AMD environments [9-11]. In acidic environments, microbial community diversity and composition have been thoroughly investigated [12,13] perhaps because it regulates other geochemical factors. The pH is frequently regarded as the most important explanatory variable for regulating microbial distribution across several acid mine drainage (AMD) sites [14]. However, in acidic conditions, pH is not the sole factor affecting the composition of the microbial population. Moreover, iron content and speciation, dissolved oxygen, temperature, organic carbon, sulphate, salinity, conductivity, C:N ratio and other metal concentrations are the geochemical factors identified as the major determinants which affect the makeup of the microbial community composition [15-21]. Microbial community dynamics can also be impacted by hydrodynamic factors such as velocity, retention time, turbulence, and precipitation patterns [22,23]. There is still a lot of uncertainty regarding the relative significance of these factors in determining microbial communities in natural environments. Kuang et al. (2013) and Wang et al. (2020) have attempted to systematically explore microbial geographical patterns by simultaneously taking into account spatial distance and contemporary environmental variations [3,24]. Rapid technical improvements in bacterial identification procedures have resulted in a formidable array of approaches for detecting, identifying, and distinguishing bacteria [25]. AMD systems were surveyed for microbial diversity using 16S rRNA genes for the first time in the mid-1990s [26,27]. Moreover, molecular diversity inventories of AMD microbes have been carried out in a variety of acidic environments in diverse geographic locations, including Svalbard [28], Iron Mountain in California, USA [29], Southern Portugal [30], Paryas Mountain, UK [31] and Southwestern Spain [32]. The majority of these studies have examined a limited number of samples from a single mining environment, and clone library analysis provides relatively limited sequencing depth compared to other approaches. Despite the technological advances, culture-based strategies are still necessary to obtain information regarding adaptation, the role of microorganism to create an environment, and their application as it grows in extreme condition, they can produce valuable enzymes, as well as determine whether the phenotypic selection is occurring or not [33]. Detecting intrastrain diversity is therefore often complemented by colony morphology characterization [34]. Microorganisms grow and form colonies on nutrient agar medium surfaces using routine culturing techniques, and the appearance of these colonies enables scientists to distinguish between genera or even species [34,35]. The finding of identical colony patterns among different samples by using this technique and likewise the existence of distinct characteristics when cultivating one variety under similar conditions are two of its most remarkable properties [36]. The identification of colony morphologies presents a significant difficulty for researchers due to the enormous number of accountable patterns [37]. In India, Gujarat is the highest lignite-producing state after Tamil Nadu. In Gujarat, mineral mines run under the authorities of Gujarat which is Gujarat Mineral Development Corporation (GMDC). Encouragement of knowledge regarding bacterial communities in the mining ecosystem of south Gujarat is the main objective of this research. To achieve that we have isolated the indigenous heterotrophic microbiota from Rajpardi lignite mine sites located in the south part of Gujarat state, India. The isolation and characterization of the native microbial flora of the lignite mine ecosystem were done. Microbial population diversity in samples was measured by diversity indices such as the Shannon-Weaver index and Simpson index. The similarity and dissimilarity of the population within the site were calculated by alpha diversity indices using PAST software.

MATERIAL AND METHODS

Site description and sample collection

A total of 9 water samples and 3 sediment samples were collected from the Rajpardi lignite mine. The mine is a Gujarat government undertaking and is located at 21°43'27.2" N, 73°12'50.0" E at Rajpardi, Gujarat, India. Rajpardi mine is an opencast lignite mine (Figure 1) and contains approximately 1% of sulfur content. Water samples were collected in sterile plastic containers and brought to the laboratory as soon as possible. Similarly, sediment samples were collected at the same sites and transferred into sterile polyethene bottles

with a sterile plastic scoop. Both water and sediment samples were kept in the refrigerator at 4 °C till analyzed.

Physicochemical analysis

The physicochemical analysis of samples was conducted *in situ* and *ex-situ* including pH, temperature, conductivity, redox potential (Eh), salinity and total dissolved solids (TDS), both at the time of sample collection and also once brought to the laboratory using respective portable meters (Eutech, Singapore).

Cultivation of bacterial varieties (morphotypes) and their diversity analysis

Isolation and enumeration of microbial diversities were obtained by the standard spread plate method. Serial dilutions of each sample were made in sterile distilled water as per requirement. For the sediment sample, 1 g of wet weight was suspended in 10 mL of sterile distilled water and vigorously mixed, followed by serial dilutions. From each dilution, 0.1 mL of the sample was evenly spread on each growth medium plate namely Nutrient agar, High plate count agar, Yeast extract agar, Actinomycetes agar and Czapek Dox agar medium for obtaining heterotrophic counts. Samples were inoculated on various media with varied pH (4 and 7). All the plates were incubated for 72 h at 32±3 °C. The total viable count was calculated in terms of CFU/mL and CFU/g from water and sediment samples, respectively. All the media used in the study were dehydrated media procured from Hi Media, India.

Statistical data analysis

Based on CFU obtained on plates (a total of 5 media of 2 different pH were used), statistical analysis was conducted to estimate the variance between the samples and isolates. Following the total count, relative density and relative frequency were calculated according to Eq. 1 and 2.

$$Relative\ density\ (Rd) = \frac{No.\ of\ individual\ of\ a\ species\ at\ a\ site}{Total\ number\ of\ individuals\ at\ site} \times 100 \quad (1)$$

$$Relative\ frequency\ (Rf) = \frac{No.of\ samples\ in\ which\ species\ present}{Total\ number\ of\ samples} \times 100 \quad (2)$$

To analyze the total variance within the sites and samples, other statistical analyses including bacterial diversity indices calculations, multivariate data analysis, principal component analysis (PCA) and Neighbour-joining analysis were conducted using PAST software version 4.03 [38].

RESULT AND DISCUSSION

Physicochemical analysis

There were 9 water samples and 3 sediment samples collected from different locations of the Rajpardi lignite mine in Gujarat, India. All samples were freshly collected in 2021 and as shown in Table 1, the temperature of samples during collection was in a range between 24.9 to 33.1 °C. The pH values of water samples were in ranged between 2.85 to 8.54. The lowest pH value was of sample 4 of Bhuri sump and the highest pH value was found for Amod mine site samples 2 and 1 which were more than 8.0. Surface weathering of spoils and accumulation of geogenic salts such as sulfates, magnesium, chlorides, and sodium chlorides cause salinity in coal mine environments [39]. Electrical conductivity (EC) is the measure of liquid capacity to conduct an electric charge and both the values of EC and total dissolved solids (TDS) were correlated [40,41]. Upon measurement, its ability depends on the concentration of dissolved ions, ionic strength, and temperature. TDS is commonly employed to determine the concentration of dissolved ions [42]. The level of TDS reflects the presence of inorganic salts and trace amounts of organic matter in water, whereas EC measures the capacity of water to conduct electrical current [43,44]. The sources of material in TDS and EC can come from nature, i.e., geological conditions, water resources, and human activities, like domestic and industrial waste and also from agriculture [45-47]. The conductivity values of samples were in the range of 0.47 to 2.83 mS/cm. TDS and salinity were in the range between 0.34 to 1.91 ppt and 233 to 1500 mg/L respectively (Table 1).

Microbial count

Table 2 depicts the number of isolates obtained on different media namely, Nutrient agar, Yeast extract agar, High plate count agar, Actinomycetes agar and Czapek dox agar of respective pH. The different microbiological medium was selected depending on the pH of the samples collected from the sites. Irrespective of the mine sample and pH, 187 heterotrophic microbial morphotypes were cultivated, including 63 isolates from water samples and 124 isolates from sediment samples. The highest microbial growth obtained from sediment samples in terms of colonies (72) was obtained from different media containing 7 pH followed by pH 4 (52). The result indicates that the sediment sample of the mine site was highly supportive of microbial growth. The highest colony count was obtained from sediment samples on YEA medium followed by CDA which indicates that mine is also a good habitat for fungi, yeast, actinomycetes and bacteria.

Morphologically and culturally different 12 fungal isolates were obtained from various agar media. Based on their microscopic morphological observation showed that out of 12 fungal isolates most of the isolates belong to the *Aspergillus*, *Mucor* and *Penicillium* whereas only one isolate belonged to the *Rhizopus* genus. The cultural characteristics of all the fungal isolates are depicted in Table 3.

Morphological characterization of isolates

The morphology of colonies could be a sign of phenotypic variation, a key adaptive strategy used by bacteria to deal with environmental stressors. The impact of colony morphology on morphogenesis has rarely been investigated in depth using experimental circumstances. Several variables, including sample collection time, collection site, pH, type of medium used, and physicochemical factors, are investigated in this study to determine their impact on bacterial colony morphology. Isolates of various sample types that had been cultured on various media shared traits and revealed both similarities and distinctions. The findings showed that all variables that influenced colony morphogenesis were found, with varying sizes, shapes, margins, textures, elevations and pigmentations. The variables that had the greatest impact on colony differentiation in both water and sediment samples were collection time, site, and medium composition. The maximum number of bacterial varieties (morphotypes) were cultivated on YEA irrespective of sediment sample and at medium pH 4 and 7 used for cultivation, while the lowest bacterial count was obtained in AA medium (Table 4). Several studies confirm that the increase in the relative number of microorganisms in sediment samples compared to the water samples pointed to some relation of microbiota with nutrients present in the sample [48-50]. On nutrient agar medium bacterial isolates, 77% were gram-negative and 23% were gram-positive. Isolates obtained from sediment samples on Czapek Dox agar medium were gram-negative, non-motile and non-spore formers. Our findings are in concordance with the previous studies [51-54] the majority of the bacterial isolates from the mine site obtained on different media were gram-negative, non-motile and non-spore formers. Morphologically different 159 bacterial isolates were isolated from the collected samples. The colony size and shape of the isolates ranged from 0.2 to 8 mm and round to irregular but were different in other traits, such as the margin and elevation. While 2 isolates of sediment samples obtained on CDA were star-shaped. Out of 159 bacterial varieties, 15% of the isolates were able to produce varieties of pigments while the rest of them gave non-pigmented colonies. Pigmented colonies showed orange, pink, yellow, brown, reddish pink, white and cream whereas, the majority of the colonies were non-pigmented. All isolates obtained on the HPC medium were smooth in texture, moist consistency with nil pigmentation. They were ranging in colour from white to yellow whereas differences were observed in opacity and pigmentation (translucent to opaque opacity with no pigmentation to white (detailed colony morphology data not shown).

Richness and evenness

The species diversity of cultivable bacteria of Rajpardi mine samples was measured based on species number and individual counts (relative density and relative frequency). Isolates 2 and 28 showed the highest relative frequency of 72% because these isolates were found in 6 different samples including WS1, WS2, WS4, SS1, SS2 and SS3 while the majority of the isolates were found in just one sample and had the lowest relative frequency of 12%. The results of Table 3 correlate with Table 1, isolate number 2 was able to survive at lower as well as higher pH while isolate 28 was able to survive at lower pH only because the presence of these isolates was found in the samples with lower pH. The majority of the isolates 20 and 105 had a relative frequency of 48% and showed their presence in four different samples. Similarly, isolates 18, 31, 36, 60, 68, and 96 had a relative frequency of 36% as their presence in three different samples (Table 5). The majority of the isolates of the sediment sample have very less relative frequency as most of the isolates were present in only singles samples indicating that within the mine ecosystem microbiota distribute in diverse patterns. The similarity in terms of Dice Matrix indices, as most of the bacterial morphology is similar due to similar pH range, sample SS3 showed the highest of 30% similarity with WS4 but 100% similarity was not found among any of the samples (Table 6). The sediment samples 2 and 1 was having the highest number of varieties (Figure 2) and thus, had the highest Shannon index of 3.197 which indicates a community with many taxa. Similar results are also observed in Figure 2. While, water sample 3, on the other hand, had the lowest Shannon index of 0.54, indicating a population of very fewer taxa (Table 7).

PCA analysis and neighbour-joining analysis

As shown in Figures 3 and 4, the Principal component analysis (PCA) and Neighbour joining graph generated through PAST software were used to determine the richness data of isolated replicates from water and sediment samples collected from the Rajpardi mining sites. The first Principal component (PC1) has a variation of 17.31%, meanwhile, the second Principal component (PC2) has a variance of 14.98%. In general, the scattering pattern obtained in PCA showed similarity with the clustering pattern of the Neighbour-joining tree. The figure depicts how the water and sediment samples diverged substantially in

terms of microbial morphology and sample type of mine. The greater variance between sites in terms of replicates morphology is greater than sample type, as shown in the plot, where samples from the same location (sump) cluster together. This reveals that the physicochemical composition of the sites drove diversity variation. When the PCA of the selected isolates was examined, it was revealed that the first PC was able to distinguish the samples of the Rajpardi mine site such as WS1, WS2 and WS3 from samples WS5, WS7 and WS8. In accordance, the Rajpardi mine site in terms of morphology similarity shows WS8 and WS7 maximum distance from the SS1 sample, where SS2 sample replicates show the highest microbial growth with varieties on solid media. The similarity in the result found in the Neighbour-joining graph is that sediment sample 3 is completely distinct from the rest of the samples in terms of microbial diversity. Moreover, the sediment samples of Rajpardi sites were closely placed in one group in PC2, as they showed less variation and comparatively more evenness in terms of analogous morphological characteristics than the first PC as very few isolates were obtained from these samples. A total of 5 major clusters of 187 isolates are formed in the Neighbour-joining plot. A bootstrap value in the Neighbour-joining plot is shown at each node (based on 1000 bootstrap resampling). In evolutionary terms, a horizontal line represents the evolutionary distance between two nodes, and a branch point along its length represents the divergence between samples. According to the PCA plot and Neighbour joining graph, Rajpardi mine water and sediment samples revealed a most considerable divergence from one another, encouraging us to conclude that the replicates had diversity at different pH levels, as both are present at different clades.

Table 1. Physicochemical characteristics of the collected samples from Rajpardi lignite mine.

Sample code	Mine site	Physicochemical parameter				
		pH	Conductivity (mS)	TDS (ppt)	Salinity (ppm)	Temperature (°C)
Water sample						
WS1	Amod	8.29	0.47	0.34	233	28.2
WS2	Amod	8.54	0.57	0.41	286	28.2
WS3	Bhuri	2.87	1.48	1.05	765	28.2
WS4	Bhuri	2.85	1.37	0.97	696	28.2
WS5	Maljipura	7.78	0.91	1.00	725	28.2
WS6	Amod	3.05	0.61	0.43	302	33.1
WS7	Amod	3.14	0.56	0.40	280	33.1
WS8	Amod	2.91	2.83	1.91	1500	33.1
WS9	Amod	2.9	2.73	1.83	1420	33.1
Sediment sample						
SS1	Amod	3.11	0.99	0.79	877	33.1
SS2	Amod	3.47	0.98	0.69	717	33.1
SS3	Amod	3.07	1.41	0.77	983	33.1

Table 2. Number of microbial varieties obtained on various media from different samples

Sample	Number of bacterial varieties (morphotypes)									
	Medium (pH 7)					Medium (pH 4)				
	NA	HPCA	YEA	AA	CDA	NA	HPCA	YEA	AA	CDA
WS1	1	0	1	0	0	1	0	0	0	2
WS2	2	0	1	0	0	0	0	1	0	0
WS3	1	0	0	0	2	0	0	0	0	1
WS4	1	0	0	0	1	0	0	1	0	3
WS5	2	2	0	0	1	0	1	0	0	0
WS6	2	1	1	1	0	0	0	2	1	0
WS7	2	2	2	0	0	0	3	2	0	0
WS8	3	0	0	1	0	0	1	1	0	3
WS9	1	4	0	1	1	0	2	1	0	0
SS1	7	7	18	6	4	2	0	11	0	6
SS2	8	0	14	5	2	2	6	13	1	6
SS3	0	0	1	0	0	0	0	3	1	1

(NA: Nutrient agar; HPCA: High plate count agar; YEA: Yeast extract agar; AA: Actinomycetes agar and CDA: Czapek Dox agar)

Table 3. Cultivated fungal isolates from Rajpardi water and sediment samples and their tentative identification based on microscopic observation

Medium	Sample	Medium pH	Isolate no.	Primary identification of fungi
Nutrient agar	Water	7	WS21	*Aspergillus*
		4	WS11	*Aspergillus*
	Sediment	7	SS22	*Rhizopus*
			SS26	*Aspergillus*
		4	SS11	*Rhizopus*
Yeast extract agar	Water	4	WS61	*Penicillium*
			WS62	*Aspergillus*
			WS91	*Mucor*
	Sediment	7	SS213	*Mucor*
		4	SS12	*Aspergillus*
			SS17	*Aspergillus*
			SS19	*Penicillium*
			SS110	*Aspergillus*
			SS21	*Aspergillus*
			SS213	*Mucor*
			SS32	*Mucor*
High plate count agar	Water	7	WS94	*Mucor*
		4	WS51	*Mucor*
			WS73	*Aspergillus*
	Sediment	7	SS17	*Mucor*
R2 agar	Water	7	WS61	*Mucor*
			WS91	*Penicillium*
		4	WS61	*Penicillium*
	Sediment	7	SS22	*Penicillium*
Czapek Dox agar	Water	7	WS41	*Penicillium*
		4	WS42	*Aspergillus*
	Sediment	7	SS22	*Aspergillus*
		4	SS31	*Aspergillus*

Table 4. Primary characteristics of the microbial isolates cultivated on different solid media

Sample	Medium pH	Isolates no.	Gram reaction	Motility	Spore formation
Nutrient agar	7	WS11, WS51, WS52, WS61, SS13, SS25, SS28	+	Motile	+
		WS22, WS31, WS41, WS62, WS71, WS72, WS81, WS82, WS83, WS91, SS11, SS12, SS15, SS16, SS17, SS21, SS23, SS24	-	Non-motile	-
		SS14	+	Non-motile	-
		SS27	-	Motile	-
	4	SS12	-	Motile	-
		SS21, SS31	+	Non-motile	-
Yeast extract agar	7	WS11, WS61, WS71, WS72, SS11, SS12 SS13, SS15, SS16, SS18, SS111, SS112, SS113, SS114, SS115, SS116, SS117, SS118, SS22, SS23, SS24, SS25, SS27, SS29, SS211, SS214, SS212	-	Non-motile	-
		SS110	+	Non-motile	-
		WS21, SS17, SS21, SS26, SS28, SS210, SS31	+	Motile	+
		SS14, SS19	-	Motile	-
	4	WS21, WS41, WS81, SS14	+	Non-motile	-

		WS71, WS72, SS15, SS16, SS111, SS23, SS24, SS25, SS26, SS27, SS28, SS29, SS210, SS211, SS212, SS31, SS33	-	Non-motile	-
		SS11, SS13, SS18	+	Motile	+
		SS22	-	Motile	-
High plate count agar	7	WS51, WS52, WS72, WS91, WS92, WS93, SS12, SS14, SS16	-	Non-motile	-
		WS61, WS71, SS11, SS13, SS15	+	Motile	+
	4	WS71, WS72, WS91, WS92, SS21, SS22, SS23, SS24, SS25, SS26	-	Non-motile	-
		WS81	+	Motile	+
R2 Agar	7	WS81, SS12, SS13, SS14, SS15, SS16, SS21, SS23, SS24	-	Non-motile	-
		SS11	+	Non-motile	-
		SS25	+	Motile	+
	4	SS21, SS31	+	Non-motile	-
Czapek Dox Agar	7	WS31, WS32, WS91, SS11, SS12, SS13, SS14, SS21	-	Non-motile	-
		WS51	+	Motile	+
	4	WS11, WS31, WS41	+	Non-motile	-
		WS12	+	motile	+
		WS43, WS81, WS82,WS83, SS11, SS12, SS13, SS14, SS15, SS16, SS21, SS22, SS23, SS24, SS25, SS26	-	Non-motile	-

WS= isolates from water sample; SS= isolates from sediment sample

Table 5. Relative density and relative frequency of cultivable bacteria from Rajpardi mine samples

Isolate no.	Relative density												Relative frequency
	Total microbial count												
	Water sample (× 10^3 CFU/ml)									Sediment sample (× 10^3 CFU/g)			
	WS1	WS2	WS3	WS4	WS5	WS6	WS7	WS8	WS9	SS1	SS2	SS3	
1							0.32						12
2	7.1	3.8		7.69						0.28	0.15	1.69	72
3		85.1	80.5										24
4				23.1									12
5					2.8								12
6					2.8	8.3							12
7						16.7							12
8	28.6												12
9	50						0.32						12
10								2.7					12
11								5.5					12
12								2.7					12
13									1.54				12
14		7.4											12
15						8.3							12
16							23.9						12

17							72.1						12
18					59.2								36
19					32.4								12
20						16.7				0.56	0.29	1.69	48
21							0.16	10.8		0.56			12
22							0.48						12
23									1.54				12
24									46.1				12
25									4.62				12
26					1.41	16.7			1.54		0.29		12
27								64.8					12
28				15.4		8.33			1.54	0.28	0.15	15.3	72
29			0.91										12
30			18.4										12
31	7.1				1.4						0.45		36
32									18.5				12
33										20.9			12
34										0.56			12
35										1.67			12
36										0.56	0.59	69.5	36
37										1.39			12
38										0.28			12
39										0.28			12
40											0.15		12
41										4.19	0.29		24
42											0.15		12
43											22.3		12
44											0.29		12
45											0.29		12
46											0.74		12
47											0.45		12
48										0.28			12
49										0.56			12
50										0.56			12
51										2.23	4.17		24
52										1.39			12
53										0.56			12
54										0.56			12
55										0.28			12
56										0.56			12
57										0.28			12
58										0.56	0.15		24
59										0.84			12
60										2.23	0.59	1.69	36

61										3.35			12
62										1.39			12
63										0.56			12
64										0.28			12
65										0.56			12
66											0.15		12
67											2.38		12
68										0.84	2.52	6.78	36
69											0.29		12
70											0.45		12
71											0.15		12
72											0.29		12
73											0.29		12
74											0.15		12
75											0.15		12
76											0.45		12
77											0.29		12
78											0.15		12
79										0.28	0.74		24
80										5.59			12
81										0.28			12
82										0.28			12
83										0.56			12
84										4.19			12
85										5.87			12
86										2.51			12
87										3.63			12
88										0.56			12
89										9.78			12
90											0.59		12
91											0.74		12
92											0.74		12
93										2.79	0.59		24
94										3.35	37.2		24
95										0.28			12
96				7.69						0.56	1.49		36
97											0.29		12
98		3.7											12
99				38.5									12
100						8.33							12
101						16.6							12
102							0.48						12
103							0.95				1.04		24
104								2.7					12

105									1.54	0.28	0.15	3.39	48
106							0.95						12
107							0.16				0.29		24
108							0.16						12
109									3.08				12
110									20		0.74		24
111	7.1												12
112			0.23										12
113				7.69									12
114								5.41					12
115								2.7					12
116								2.7					12
117										1.12			12
118											0.15		12
119											0.15		12
120											0.29		12
121											0.29		12
122											2.97		12
123											0.59		12
124										0.28			12
125										0.56			12
126											0.15		12
127											0.15		12
128											0.15		12
129											0.74		12
130											0.15		12
131											0.74		12
132											2.23		12
133											4.16		12
134											0.45		12
135											0.15		12
136											0.15		12
137										0.28			12
138										1.11			12
139										0.28			12
140										4.47			12
141										0.28			12
142										1.11			12
143											0.15		12
144											2.23		12
145											0.15		12

Table 6. Dice similarity or distance matrix

	WS1	WS2	WS3	WS4	WS5	WS6	WS7	WS8	WS9	SS1	SS2	SS3
WS1	1	0.22	0	0.18	0.18	0	0.12	0	0	0.03	0.06	0.17
WS2	0.22	1	0.25	0.2	0	0	0	0	0	0.03	0.03	0.18
WS3	0	0.25	1	0	0	0	0	0	0	0	0	0
WS4	0.18	0.2	0	1	0	0.14	0	0	0.12	0.09	0.08	0.30
WS5	0.18	0	0	0	1	0.28	0	0	0.12	0	0.05	0
WS6	0	0	0	0.14	0.28	1	0	0	0.22	0.06	0.08	0.26
WS7	0.12	0	0	0	0	0	1	0.1	0	0.02	0.05	0
WS8	0	0	0	0	0	0	0.1	1	0	0.03	0	0
WS9	0	0	0	0.12	0.12	0.22	0	0	1	0.06	0.11	0.23
SS1	0.03	0.03	0	0.09	0	0.06	0.02	0.03	0.06	1	0.23	0.22
SS2	0.06	0.03	0	0.08	0.05	0.08	0.05	0	0.11	0.23	1	0.20
SS3	0.16	0.18	0	0.30	0	0.26	0	0	0.23	0.22	0.20	1

Table 7. Alpha diversity indices of Rajpardi mine site samples

Sample	Total no. of individuals	Dominance	Simpson index (D)	Shannon index (H)	Evenness	Fisher's alpha	Berger Parker	Chao1 index
WS1	1400	0.3469	0.6531	1.27	0.7122	0.6516	0.5	5
WS2	2700	0.7339	0.2661	0.5735	0.4436	0.4611	0.8519	4
WS3	43500	0.6813	0.3187	0.5434	0.4305	0.3401	0.8046	4
WS4	1300	0.2426	0.7574	1.586	0.8138	0.8133	0.3846	6
WS5	13500	0.2521	0.7479	1.491	0.6343	0.7105	0.3111	7
WS6	1000	0.16	0.84	1.887	0.9425	1.015	0.2	7
WS7	62700	0.5772	0.4228	0.7857	0.1994	0.9954	0.7209	11
WS8	3700	0.4419	0.5581	1.325	0.4179	1.109	0.6486	9
WS9	6300	0.3096	0.6904	1.447	0.5311	0.904	0.4762	8
SS1	34800	0.07698	0.923	3.197	0.4367	6.525	0.2155	56
SS2	67100	0.1965	0.8035	2.476	0.195	6.612	0.3726	61
SS3	5900	0.5128	0.4872	1.044	0.4059	0.7842	0.6949	7

Figure 1. (a) Rajpardi lignite mine site; (b) ETP plant at Rajpardi

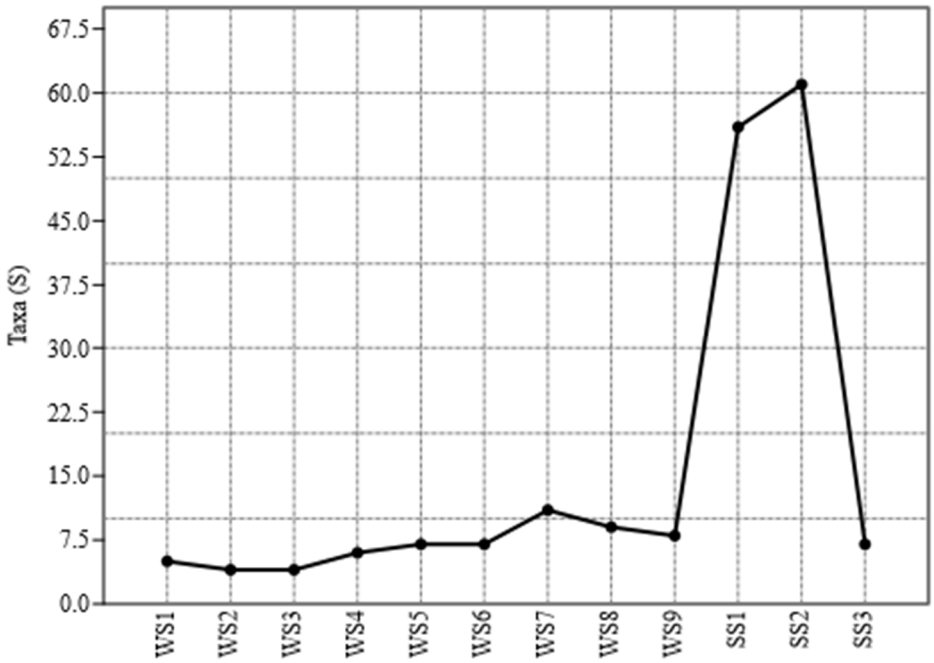

Figure 2. Number of microbial taxa present in the studied lignite mine samples

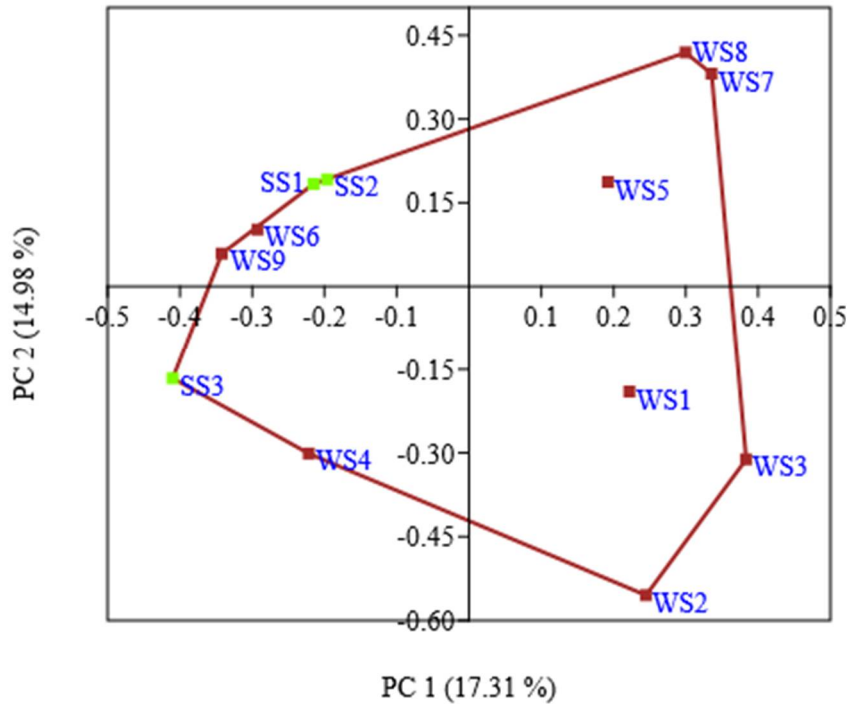

Figure 3. PCA plot of variance between samples of the studied lignite mine

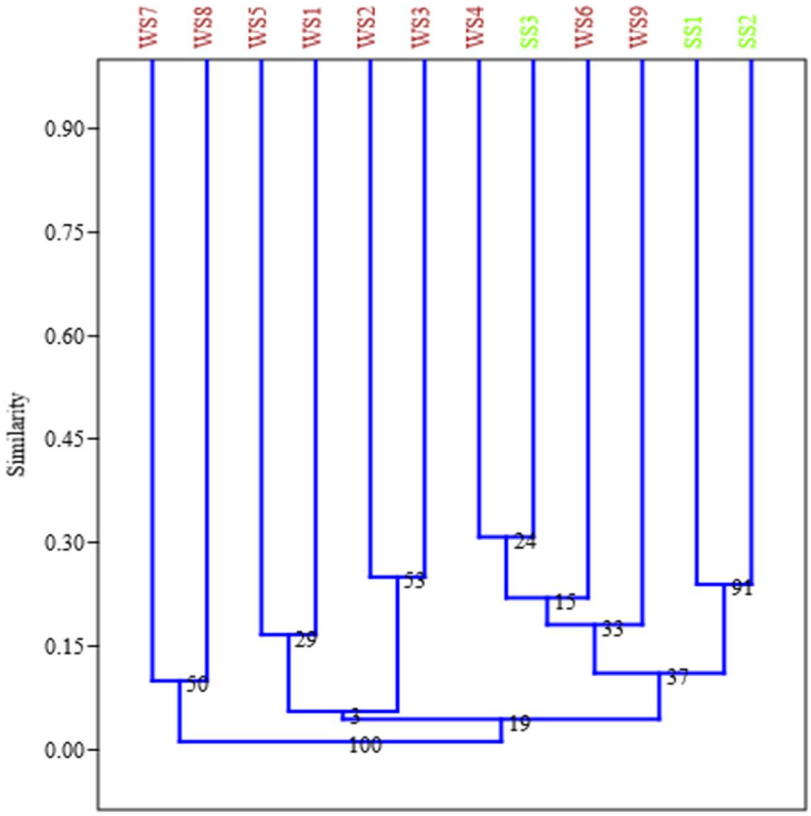

Figure 4. Neighbour-joining plot showing the grouping of samples based on the cultivable microbial morphology data

CONCLUSION

The microbial communities at this location were diverse groups driven by a plethora of variables. The findings of the study suggest that the majority of the bacterial isolates from the mine site obtained on different media were gram-negative, non-motile and non-spore formers. Concerning physicochemical attributes, the locations of mine sites were diverse from one another. These findings indicate that the physicochemical features may have influenced the formation of microbiomes. In water samples, the heterotrophic bacterial isolates decrease whereas in sediment samples, the count increases. The sediment sample was having the highest number of varieties and thus, had the highest Shannon index of 3.197 which indicates a community with many taxa. The PCA results revealed that the diversity between water and sediment samples of the mine site was comparable, and they shared common taxa in terms of morphology. Here, the diversity data of those indigenous microorganisms particularly bacteria are shown which are cultivable in the laboratory on simple media but, the whole community including identities of non-cultivable isolates and potential roles need to be elucidated, using a culture-independent approach.

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COMPETING INTERESTS

The authors have declared that no competing interest exists.

REFERENCES

1. Mony, C., Vandenkoornhuyse, P., Bohannan, B.J., Peay, K., & Leibold, M.A. (2020). A landscape of opportunities for microbial ecology research. Front. Microbiol., 11:561427.
2. Kuang, J.L., Huang, L.N., Chen, L.X., Hua, Z.S., Li, S.J., Hu, M., Li. J.T., & Shu, W.S. (2013). Contemporary environmental variation determines microbial diversity patterns in acid mine drainage. ISME J., 7(5):1038-1050.
3. Vass, M., Székely, A.J., Lindström, E.S., & Langenheder, S. (2020). Using null models to compare bacterial and microeukaryotic metacommunity assembly under shifting environmental conditions. Sci. Rep., 10(1):1-13.

4. Sheng, Y., Bibby, K., Grettenberger, C., Kaley, B., Macalady, J.L., Wang, G., & Burgos, W.D. (2016). Geochemical and temporal influences on the enrichment of acidophilic iron-oxidizing bacterial communities. Appl. Environ. Microbiol., 82(12):3611-3621.
5. Karagüzel, C., Ören, Ö., Şahbaz, M., Canıeren, Ö., Demir, U., & Şahbaz, O. (2020). Prediction of acid mine drainage potential of dump sites by using static tests: an application on lignite mine. Arab. J. Geosci., 13:1-14.
6. Chen, G., Ye, Y., Yao, N., Hu, N., Zhang, J., & Huang, Y. (2021). A critical review of prevention, treatment, reuse, and resource recovery from acid mine drainage. J. Clean. Prod., 329:129666.
7. Hedrich, S., & Schippers, A. (2021). Distribution of acidophilic microorganisms in natural and man-made acidic environments. Curr. Issues Mol. Biol., 40(1):25-48.
8. Bruneel, O., Mghazli, N., Sbabou, L., Héry, M., Casiot, C., & Filali-Maltouf, A. (2019). Role of microorganisms in rehabilitation of mining sites, focus on Sub Saharan African countries. J. Geochem. Explor., 205:106327.
9. Wei, Y., Wang, F., Gao, J., Huang, Y., Ren, W., & Sheng, H. (2021). Culture-dependent and culture-independent characterization of bacterial community diversity in different types of sandy lands: the case of Minqin County, China. BMC Microbiol., 21(1):1-15.
10. Sorgen, A., Johnson, J., Lambirth, K., Clinton, S.M., Redmond, M., Fodor, A., & Gibas, C. (2021). Characterization of environmental and cultivable antibiotic-resistant microbial communities associated with wastewater treatment. Antibiotics., 10(4):352.
11. Liu, Y.H., Mohamad, O.A.A., Gao, L., Xie, Y.G., Abdugheni, R., Huang, Y., Li, L., Fang, B.Z., & Li, W.J. (2023). Sediment prokaryotic microbial community and potential biogeochemical cycle from saline lakes shaped by habitat. Microbiol. Res., 270:127342.
12. Shang, Y., Wu, M., Zhang, J., Meng, H., Hong, J., Hao, X., Lead, J.R., & Wang, X. (2023). Nutrient enhanced reclamation promoted growth, diversity and activities of carbon fixing microbes in a coal-mining subsistence land. Soil Sci. Environ., 2(1).
13. Chen, J., Nan, J., Xu, D., Mo, L., Zheng, Y., Chao, L., Qu, H., Guo, Y., Li, F., & Bao, Y. (2020). Response differences between soil fungal and bacterial communities under opencast coal mining disturbance conditions. Catena., 194:104779.
14. Wolkersdorfer, C. (2022). Active Treatment Methods for Mine Water. Mine Water Treatment–Active and Passive Methods. Springer, Berlin Heidelberg, p.95-149
15. Zhang, X., Tang, S., Wang, M., Sun, W., Xie, Y., Peng, H., Zhong, A., Liu, H., Zhang, X., Yu, H., Giesy, J.P., & Hecker, M. (2019). Acid mine drainage affects the diversity and metal resistance gene profile of sediment bacterial community along a river. Chemosphere., 217:790-799.
16. Sun, P., Wang, Y., Huang, X., Huang, B., & Wang, L. (2022). Water masses and their associated temperature and cross-domain biotic factors co-shape upwelling microbial communities. Water Res., 215:118274.
17. Koner, S., Tsai, H.C., Chen, J.S., Hussain, B., Rajendran, S.K., & Hsu, B.M. (2022). Exploration of pristine plate-tectonic plains and mining exposure areas for indigenous microbial communities and its impact on the mineral-microbial geochemical weathering process in ultramafic setting. Environ. Res., 214:113802.
18. Li, J., Wang, X., Wu, J.H., Sun, Y.X., Zhang, Y.Y., Zhao, Y.F., Huang, Z. & Duan, W.H. (2023). Climate and geochemistry at different altitudes influence soil fungal community aggregation patterns in alpine grasslands. Sci. Total Environ., 881:163375.
19. Kim, H.S., Park, K., Jo, H.Y., & Kwon, M.J. (2023). Weathering extents and anthropogenic influences shape the soil bacterial community along a subsurface zonation. Sci. Total Environ., 876:162570.
20. Shu, W.S., & Huang, L.N. (2022). Microbial diversity in extreme environments. Nat. Rev. Microbiol., 20(4):219-235.
21. Kiran, M.G., Pakshirajan, K., & Das, G. (2017). An overview of sulfidogenic biological reactors for the simultaneous treatment of sulfate and heavy metal rich wastewater. Chem. Eng. Sci., 158:606-620.
22. Díaz, J.A., Castillo, D., Oyarzún, R., Briso, A., Montecinos, M., & Pastén, P. (2023). The dynamics of arsenic and copper in solid and aqueous phases in reactive confluences receiving acid drainage: The role of turbidity and particle size. Environ. Pollut., 327:121449.
23. Opitz, J., Alte, M., Bauer, M., & Peiffer, S. (2021). The role of macrophytes in constructed surface-flow wetlands for mine water treatment: A review. Mine Water Environ., 40(3):587-605.
24. Wang, Z., Xu, Y., Zhang, Z., & Zhang, Y. (2020). Acid Mine Drainage (AMD) in abandoned coal mines of Shanxi, China. Water., 13(1):8.
25. Kumar, V., Thakur, I.S., Singh, A.K., & Shah, M.P. (2020). Application of metagenomics in remediation of contaminated sites and environmental restoration (Eds. Shah, M.P. and Sengor, S.S) Emerging Technologies in Environmental Bioremediation, Elsevier, p.197-232
26. Goebel, B.M., & Stackebrandt, E. (1994). Cultural and phylogenetic analysis of mixed microbial populations found in natural and commercial bioleaching environments. Appl. Environ. Microbiol., 60(5):1614-1621.
27. Woese, C.R., & Fox, G.E. (1977). Phylogenetic structure of the prokaryotic domain: the primary kingdoms. In: Proceedings of the National Academy of Sciences., 74(11):5088-5090.
28. García-Moyano, A., Austnes, A.E., Lanzén, A., González-Toril, E., Aguilera, Á., & Øvreås, L. (2015). Novel and unexpected microbial diversity in acid mine drainage in Svalbard (78 N), revealed by culture-independent approaches. Microorganisms., 3(4):667-694.
29. Kumar, J., Sharma, N., & Singh, S.P. (2023). Genome-resolved metagenomics inferred novel insights into the microbial community, metabolic pathways, and biomining potential of Malanjkhand acidic copper mine tailings. Environ. Sci. Pollut. Res. Int., 30(17):50864-50882.

30. Ettamimi, S., Carlier, J.D., Cox, C.J., Elamine, Y., Hammani, K., Ghazal, H., & Costa, M.C. (2019). A meta-taxonomic investigation of the prokaryotic diversity of water bodies impacted by acid mine drainage from the São Domingos mine in southern Portugal. Extremophiles., 23:821-834.
31. Distaso, M.A., Bargiela, R., Johnson, B., McIntosh, O.A., Williams, G.B., Jones, D.L., Golyshin, P.N., & Golyshina, O.V. (2022). Microbial diversity of a disused copper mine site (Parys Mountain, UK), dominated by intensive eukaryotic filamentous growth. Microorganisms., 10(9):1694.
32. Chacon-Baca, E., Santos, A., Sarmiento, A.M., Luís, A.T., Santisteban, M., Fortes, J.C., Davila, J.M., Diaz-Crriel, J.M., & Grande, J.A. (2021). Acid mine drainage as energizing microbial niches for the formation of iron stromatolites: the Tintillo river in Southwest Spain. Astrobiol., 21(4):443-463.
33. Buchan, B.W., & Ledeboer, N.A. (2014). Emerging technologies for the clinical microbiology laboratory. Clin. Microbiol. Rev., 27(4):783-822.
34. Sousa, A.M., Machado, I., Nicolau, A., & Pereira, M.O. (2013). Improvements on colony morphology identification towards bacterial profiling. J. Microbiol. Methods, 95(3):327-335.
35. Sanders, E.R. (2012). Aseptic laboratory techniques: plating methods. J. Vis. Exp., (63):e3064.
36. Paiva, D.S., Fernandes, L., Trovão, J., Mesquita, N., Tiago, I., & Portugal, A. (2022). Uncovering the fungal diversity colonizing limestone walls of a forgotten monument in the central region of Portugal by high-throughput sequencing and culture-based methods. Appl. Sci., 12(20):10650.
37. Khalifa, N.E.M., Taha, M.H.N., Hassanien, A.E., & Hemedan, A.A. (2019). Deep bacteria: robust deep learning data augmentation design for limited bacterial colony dataset. Int. J. Reason.-based Intell. Syst., 11(3):256-264.
38. Hammer, Ø., Harper, D.A.T., & Ryan, P.D. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. Paleontol. Electron., 4 (1):1-9.
39. Park, J.H., Li, X., Edraki, M., Baumgartl, T., & Kirsch, B. (2013). Geochemical assessments and classification of coal mine spoils for better understanding of potential salinity issues at closure. Environ. Sci.: Process. Impacts., 15(6):1235-1244.
40. Rusydi, A.F. (2018). Correlation between conductivity and total dissolved solid in various type of water: A review. In: IOP Conference Series: Earth and Environmental Science, 118:012019.
41. Corwin, D.L., & Yemoto, K. (2020). Salinity: Electrical conductivity and total dissolved solids. Soil Sci. Soc. Am. J., 84(5):1442-1461.
42. Liu, S., Zhang, K., Gao, J., Yang, Y., Bai, L., & Yan, J. (2023). Research on temporal patterns of water–rock interaction in the coal mine underground reservoir based on the dynamic simulation test. ACS Omega., 8(15):13819-13832.
43. Jeyasekhar, M.P. (2022). Physico-chemical analysis of ground water samples from different taluks in Kanyakumari district, Tamil Nadu, India. J. Xi'an Shiyou Uni, Nat. Sci. Ed., 18(5):415-430.
44. Tesfaye, T., Tesfau, H., Balcha, A., & Hassen, M. (2023). Assessment of the level of some selected heavy metals and physicochemical in Abzana water samples, Kibet woreda, Ethiopia. Int. J. Environ. Sci. Technol., 20(4):4383-4390.
45. Helard, D., Indah, S., & Wilandari, M. (2021). Spatial variation of electrical conductivity, total suspended solids, and total dissolved solids in the Batang Arau River, West Sumatera, Indonesia. In: IOP Conference Series: Materials Science and Engineering, 1041(1):012027.
46. Güler, C., Kurt, M. A., Alpaslan, M., & Akbulut, C. (2012). Assessment of the impact of anthropogenic activities on the groundwater hydrology and chemistry in Tarsus coastal plain (Mersin, SE Turkey) using fuzzy clustering, multivariate statistics and GIS techniques. J. Hydrol., 414:435-451.
47. Eyankware, M.O. (2019). Hydrogeochemical assessment of chemical composition of groundwater; A case study of the Aptian-Albian aquifer within sedimentary Basin Nigeria. Global J. Environ. Res., 13(1):7-16.
48. Zhang, W., Gu, J., Li, Y., Lin, L., Wang, P., Wang, C., Qian, B., Wang, H., Niu, L., Wang, L., Zhang, H., Gao, Y., Zhu, M., & Fang, S. (2019). New insights into sediment transport in interconnected river–lake systems through tracing microorganisms. Environ. Sci. Technol., 53(8):4099-4108.
49. Liu, Z., Iqbal, M., Zeng, Z., Lian, Y., Zheng, A., Zhao, M., Li, Z., Wang, G., Li, Z., & Xie, J. (2020). Comparative analysis of microbial community structure in the ponds with different aquaculture model and fish by high-throughput sequencing. Microb. Pathog., 142:104101.
50. Zhang, L., Delgado-Baquerizo, M., Shi, Y., Liu, X., Yang, Y., & Chu, H. (2021). Co-existing water and sediment bacteria are driven by contrasting environmental factors across glacier-fed aquatic systems. Water Res., 198:117139.
51. Muhadesi, J.B., Huang, Y., Wang, B.J., Jiang, C.Y., & Liu, S.J. (2019). *Acidibrevibacterium fodinaquatile* gen. nov., sp. nov., isolated from acidic mine drainage. Int. J. Syst. Evol. Microbiol., 69(10):3248-3255.
52. Sanchez-Andrea, I., Sanz, J.L., & Stams, A.J. (2014). *Microbacter margulisiae* gen. nov., sp. nov., a propionigenic bacterium isolated from sediments of an acid rock drainage pond. Int. J. Syst. Evol. Microbiol., 64(12):3936-3942.
53. Kishimoto, N., Kosako, Y., & Tano, T. (1991). *Acidobacterium capsulatum* gen. nov., sp. nov.: an acidophilic chemoorganotrophic bacterium containing menaquinone from acidic mineral environment. Curr. Microbiol., 22:1-7.
54. Wichlacz, P.L., & Unz, R.F. (1981). Acidophilic, heterotrophic bacteria of acidic mine waters. Appl. Environ. Microbiol., 41(5):1254-1261.