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Predictive functional profiling of 16S rRNA genes amplicons reveals bioremediation and sulfur metabolism capacity in thermophilic hot spring bacteriomes

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Thermophilic hot springs host highly specialized microbial communities critical for biogeochemical cycling and novel biotechnological applications. This study investigated the structure of the bacterial communities (bacteriomes) and predicted functional potential related to bioremediation and sulfur metabolism across three geochemically diverse soil sites within the Pharaoh's Bath Hot Springs ecosystem in South Sinai, Egypt. These sites were categorized by distinct thermal profiles: 70 °C (HS1), 75 °C (HS2), and 80 °C (HS3). Using 16 S rRNA gene amplicon sequencing and PICRUSt functional prediction, sequence analysis via the EzBioCloud server revealed that the HS2 site harbored the highest evenness and overall microbial diversity. Taxonomically, the HS1 and HS3 sites were dominated by *Proteobacteria*; in contrast, the HS2 site exhibited a more diverse profile, characterized by a reduced *Proteobacteria* presence and a high abundance of *Rhodothermaeota*. Predictive functional profiling identified 13 genes associated with biodegradation pathways (e.g., catechol and xylene degradation), suggesting an intrinsic genetic capacity to degrade complex aromatics and halogenated compounds across these thermal gradients. Regarding sulfur metabolism, functional predictions indicated that the HS2 site possessed the highest potential for dissimilatory sulfate reduction. Meanwhile, the HS1 site specialized in assimilatory sulfate reduction and, alongside the HS2 site, demonstrated a higher predicted capacity for sulfide oxidation. The distribution of heat-response genes varied by location: HspQ and Hsp33 were most prominent at the HS1 site, while HSP20 and DnaK reached their maximum abundance at the HS2 site. Overall, this study demonstrates the substantial intrinsic bioremediation potential of the studied bacteriomes and provides a predictive framework for understanding microbial functional potential in this system, with future studies offering opportunities to refine in situ functional validation and application.

Keywords Thermophilic microorganisms, Hot springs, Biodegradation, Metagenomics, Bioremediation

Natural hot springs are widely distributed in various locations in the world. Geothermal environments are extreme biological niches that support the life of a variety of microorganisms[1]. Geothermal springs contain diverse groups of microorganisms[2]. The microbial diversity in these geothermal environments is governed by physicochemical parameters, including water chemistry, geological origin, pressure and geothermal gradient[3,4]. Identification and classification of the extremophiles is critical, as their metabolic diversity and stable extremozymes offer significant potential for industrial and biotechnological applications[5].

In geothermally active and hypersaline sites, such as the hot springs of the Sinai Peninsula, the scarcity of organic carbon often necessitates a reliance on lithotrophic pathways. Sulfur cycling represents a critical biogeochemical engine, where specialized microbial consortia, especially sulfur-oxidizing bacteria (SOB)

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and sulfate-reducing bacteria (SRB) form intricate metabolic networks[6,7]. These microbial consortia drive the transformation of sulfur compounds across diverse oxidation states to maintain ecosystem stability[8]. These microorganisms utilize sophisticated enzymatic systems, including the sulfur oxidation (Sox) complex and dissimilatory sulfite reductase (Dsr) pathways, to thrive under poly-extreme conditions[9]. Beyond ecological maintenance, these sulfur-metabolizing extremophiles possess robust potential for bioremediation, offering novel mechanisms for the detoxification of heavy metals and the degradation of xenobiotic compounds in harsh industrial effluents[10]. The bioremediation capabilities of microbes isolated from hot spring systems have been demonstrated previously, as shown by studies identifying bacterial isolates from South African hot springs capable of producing enzymes relevant to degradation of polyaromatic hydrocarbons and dye pollutants[11], as well as bacterial isolates from Peruvian hot springs with biotechnological potential for the remediation of environments contaminated with oil, metals, and plastics[12].

Microbial mediated bioremediation of a vast array of contaminants has been proved to be a clean and cost-effective alternative to conventional treatment approaches[13]. The Pharaoh's Bath (Hammam Pharaon) ecosystem in Sinai is a rare poly-extreme habitat where temperatures reach up to 92 °C and hypersalinity converge with one of the world's highest natural sulfur concentrations[7]. Recent research emphasizes this site as a "biodiversity hotspot" for thermophilic actinomycetes and other extremophiles with significant metabolic versatility[8,14]. This unique functional potential makes the site a critical reservoir for "extremozymes" capable of degrading persistent organic pollutants, such as hydrocarbons and synthetic polymers, under harsh thermal and saline conditions that typically inhibit standard biological treatments[10,15]. Furthermore, the site's proximity to the Gulf of Suez industrial zone introduces potential anthropogenic hydrocarbons, providing a clear rationale for investigating the functional potential of resident communities in degrading persistent organic pollutants.

New-generation sequencing technologies emerged as a powerful tool in characterizing microbes in specific environments. Microbial ecology of several hot springs around the world has been studied using 16 S rRNA amplicon sequencing[16–18]. The metagenomic study has led to the cultivation-independent and comprehensive investigation of microbial and functional diversity of the ecological niches. The analysis of culture-independent metagenome sequence data is helpful in elucidating the association among taxonomic composition, their functioning, and environmental feature[19]. Metabolically diverse bacteriomes have been described in hot springs with very different physicochemical parameters[18,20], and some of their thermostable enzymes have been characterized as valuable biocatalysts with potential use in biotechnology and industry[21].

Comparative metagenomic analyses of analogous geothermal systems emphasize that temperature and sulfur availability are the primary determinants of bacteriome assembly and functional diversity. Recent studies on the Red Sea coast demonstrate that these habitats serve as evolutionary reservoirs for extremophilic genera like *Geobacillus* and *Thermus*, which possess the functional potential to degrade complex xenobiotics and precipitate heavy metals. Collectively, these findings underscore that sulfur-rich hot springs are not merely isolated geological features but are active "biorefineries" where metabolic flexibility allows for the remediation of persistent pollutants under conditions that would denature standard biological catalysts[7,10].

Despite the recognized ecological significance of the Pharaoh's Bath ecosystem, there remains a substantial knowledge gap regarding the direct correlation between microbial diversity and in situ bioremediation efficiency. While high-throughput sequencing has begun to unravel the complex taxonomic assembly of these thermal habitats, more studies are required to determine how high levels of phylogenetic diversity translate into specialized functional performance for pollutant degradation. The extreme selective pressures of high temperature and salinity likely foster unique specialist taxa whose roles in sulfur cycling and bioremediation are not yet fully understood through diversity metrics alone[7,10]. Consequently, further empirical research is essential to move beyond descriptive diversity analysis and establish the mechanistic links between community composition and the metabolic resilience required for scalable bioremediation in harsh industrial contexts.

The present study aimed to explore and characterize the bacterial communities (bacteriomes) of the Pharaoh's Bath hot spring, South Sinai, Egypt, using 16 S rRNA amplicon sequencing. This approach helps identify pollutant-degrading populations in this hot spring and assess their potential for diverse bioremediation activities. Additionally, the study focused on identifying predicted functional biomarkers that could be utilized for bioremediation purposes as further evidence for potential diverse bioremediation processes. Particular attention was also given to detecting biomarkers linked to sulfur cycling and heat stress response, highlighting their ecological and functional significance within the hot spring environment. Overall, this research aims to reveal the Pharaoh's Bath hot spring as a promising reservoir of microbial resources with substantial biotechnological and bioremediation potential.

Materials and methods

Sampling procedure and sampling site

Soil sampling was conducted at the Pharaoh's Bath (Hammam Pharaon) hot springs (29°12′24.9″ N, 32°57′35.4″ E) in the South Sinai Governorate, Egypt. Three distinct sampling sites were selected based on a perpendicular thermal gradient emerging from the primary geothermal source to capture the transition in bacteriome structure across varying heat intensities. The selected three sites represent different geographic distributions along with distinct thermal profiles: 70 °C (HS1), 75 °C (HS2), and 80 °C (HS3). At each site, soil was collected in triplicate to ensure a representative metagenomic profile. Samples were obtained from the subsurface layer at a depth of 5 cm using a sterile spatula. Approximately 50 g of soil was collected per subsample, and the three subsamples from each site were pooled at the time of collection to form a single composite sample in sterile polypropylene containers prior to DNA extraction. To maintain biological integrity, the composite samples were immediately placed in a portable icebox at 4 °C for transport and subsequently stored at − 20 °C until genomic DNA extraction was performed.

Overall, soil samples were selected over water samples because sediment/soil matrices have been reported to harbor higher microbial diversity[22] and function as long-term reservoirs of microbial communities compared to the overlying water column[23].

DNA extraction and PCR amplification of the V3-V4 region of bacterial 16 S rRNA genes

Total genomic DNA was directly extracted from soil collected at the three sampling sites using the Soil DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA, USA) following the manufacturer's protocol. The hypervariable V3–V4 region of the bacterial 16 S rRNA gene was then amplified by PCR using the universal primers 341 F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′)[24]. PCR amplification was carried out according to the Illumina 16 S rRNA Metagenomic Sequencing Library Preparation protocol at Macrogen (Daejeon, Korea). The thermal cycling program consisted of an initial denaturation at 95 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min.

Illumina miseq sequencing and bioinformatic processing of 16s rRNA gene amplicons

The PCR amplicons (~ 450 bp) obtained from the previous step were sequenced using a paired-end (2 × 300 bp) chemistry on the Illumina MiSeq platform at Macrogen (Daejeon, Korea). The resulting raw sequence reads (FASTQ format) were processed and analyzed using the Microbiome Taxonomic Profiling (MTP) pipeline[25] provided by EzBioCloud (https://www.ezbiocloud.net/contents/16smtp). Initially, Illumina paired-end reads were merged using the VSEARCH program[26], which is available under either the BSD 2-clause license or the GNU General Public License version 3.0 (https://github.com/torognes/vsearch). PCR primer sequences were subsequently removed using in-house code, followed by quality filtering to eliminate low-quality reads, such as reads with lengths < 100 bp or > 2,000 bp, average quality scores < 25, or sequences not identified as 16 S rRNA genes. Taxonomic assignment was performed by comparing sequences against the EzBioCloud 16 S database (PKSSU4.0) using VSEARCH program[26](https://github.com/torognes/vsearch), applying a 97% similarity threshold for species-level classification, with lower similarity cutoffs for higher taxonomic ranks. Chimeric sequences were identified among unmatched reads using the UCHIME algorithm[27](v4.2.52, http://drive5.com/uchime). Subsequently, operational taxonomic units (OTUs) were defined using an open-reference clustering approach, in which sequences were first aligned against reference databases, and any remaining unmatched reads were clustered using the UCLUST algorithm[28](https://drive5.com/usearch/manual/uclust_algo.html) at a 97% similarity threshold.

Alpha diversity metrics, including Good's coverage, rarefaction curves, observed OTUs, Chao1, Simpson, Shannon, and the Abundance-based Coverage Estimator (ACE), were calculated using the EZBioCloud server. ACE and Chao1 are commonly used estimators of species richness, representing the total number of species present in a sample[29,30]. In contrast, the Shannon and Simpson indices are measures of species diversity that account for both richness and evenness, reflecting not only the number of species but also the relative abundance distribution among them[31]. Beta diversity was assessed at the genus level using principal coordinate analysis (PCoA) based on Bray–Curtis distance matrices, as implemented in the EZBioCloud server. Rarefaction curve analysis was performed using the MG-RAST server[32](version v4.0.3, https://www.mg-rast.org/) under default parameters. A Venn diagram (at the genus level) was created using the InteractiVenn online tool (https://www.interactivenn.net/) to illustrate the shared and unique OTUs among the three sampling sites. The phylogenetic tree, based on the neighbor-joining method, was constructed using MEGA11 software[33](version 11, https://www.megasoftware.net/).

Functional profiling and identification of potential biomarkers related to bioremediation, sulfur cycling and heat shock response

Predictive functional biomarkers profiling was conducted using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) tool[34] integrated within the EZBioCloud platform, in combination with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database[35]. The PICRUSt analysis applied the Kruskal–Wallis H test to determine significantly different functional profiles, using a significance threshold of $P < 0.05$[36]. Heatmaps illustrating potential functional biomarkers associated with the sulfur cycle and heat shock response, as predicted by PICRUSt, were generated using the SRplot online platform (https://www.bioinformatics.com.cn/en).

Results

General characteristics of hot springs-bacteriomes

The present study employed 16 S rRNA MiSeq Illumina sequencing to investigate the bacteriome structure and composition in soil samples from Pharaoh's Bath hot spring in South Sinai, Egypt. Analysis of raw 16 S rRNA gene sequences using the EZBioCloud server yielded 42,069 to 76,564 target reads and revealed 358 to 1,419 OTUs per sample (Table 1). Good's coverage values exceeding 99% (Table 1) and plateaued rarefaction curves (Fig. 1) demonstrated sufficient sequencing depth for further analysis. The alpha diversity analysis, which measures diversity within the three identified bacteriomes (HS1, HS2, and HS3) using four indices, revealed distinct patterns of richness and evenness (Fig. 2). HS2 demonstrated the highest community evenness and overall diversity, scoring the highest on both the Shannon index and the Simpson Index. Conversely, HS3 exhibits the lowest values across all four metrics, indicating it is the least diverse, with the lowest estimated species richness and the lowest evenness. Notably, the richness-based estimators (ACE and Chao) show that HS1 and HS2 have very similar and high estimated total species numbers.

PCoA plot, designed to visualize the differences in bacteriome structure, showed clear separation of the HS1, HS2, and HS3, indicating significant compositional differences between the three sampling sites (Fig. 3A). The

Sample name	Valid reads	Valid reads percentage	OTUs	Good's coverage of library (%)
HS1	76,564	56.0%	373	99.95
HS2	55,050	63.4%	1419	99.82
HS3	42,069	59.8%	358	99.87

Table 1. Alpha diversity indices (valid reads, valid reads percentage, OTUs obtained and Good's coverage of library (%)) identified in the three bacteriomes addressed in this study.

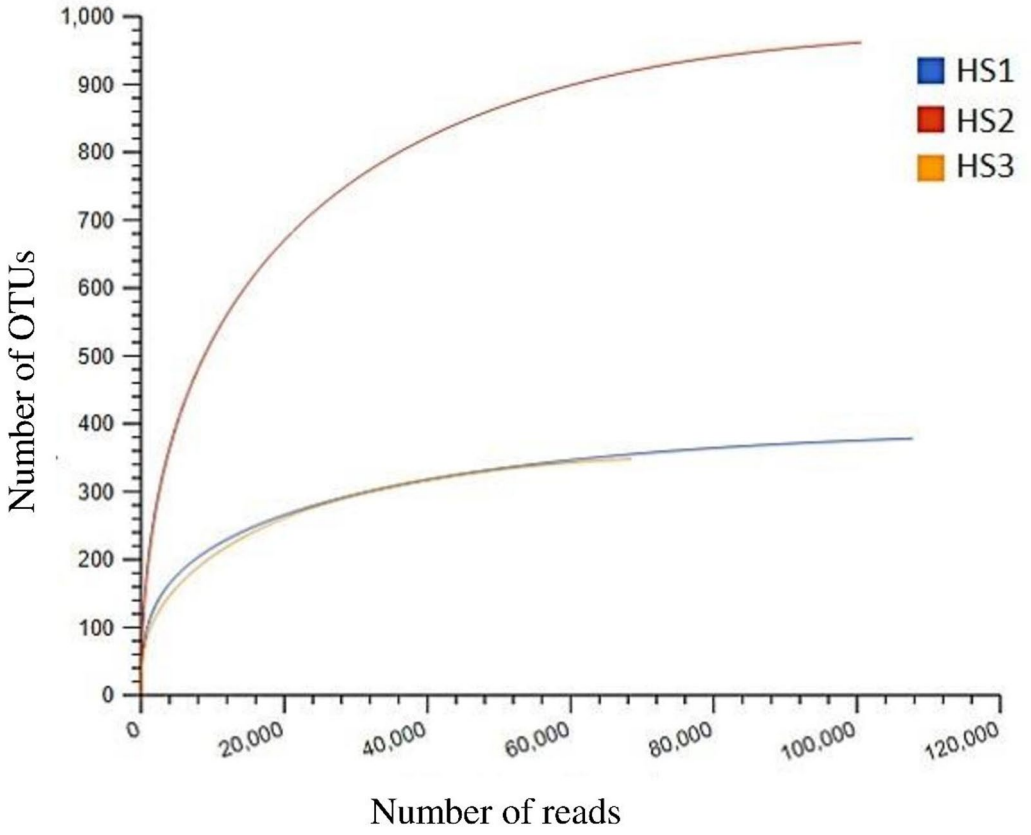

Fig. 1. Rarefaction curves for the three bacteriomes identified in this study.

greatest structural dissimilarity is observed between HS2 and HS3, indicating it has a unique bacteriome profile. Venn diagram analysis of genus-level composition (Fig. 3B) revealed that all three bacteriomes (HS1, HS2, and HS3) shared a substantial core bacteriome comprising 33 genera; however, the majority of the total 130 genera identified were unique. HS3 demonstrated the highest number of unique genera (24), followed by HS2 (17 unique genera), while HS1 had the fewest unique genera (9). Regarding pairwise overlap, HS1 and HS2 exhibited the strongest association, sharing 23 genera exclusively between them, whereas the overlap was weaker between HS1 and HS3 and weakest between HS2 and HS3, underscoring the compositional distinctiveness observed in the previous beta diversity analysis.

Taxonomic affiliation of 16 S rRNA gene sequences

The analysis of microbial relative abundance at the phylum level (Fig. 4A) across the three groups reveals HS1 and HS3 are compositionally similar but distinctly different from HS2, due to the massive dominance of Proteobacteria, which constitutes approximately 88% of HS1 and nearly 94% of HS3. Bacteroidetes were the second most abundant phylum in HS1 (5.1%) and HS3 (2.743%). HS2 presents a far more diverse and complex profile, where the dominant Proteobacteria was significantly reduced to 46%. Rhodothermaeota dominated the second position in HS2 with 20.9% relative abundance. At the genus level (Fig. 4B), the relative abundance analysis showed a dramatic compositional variation between the three communities, with HS3 being exceptionally homogeneous, dominated by *Thiomicrospira*. HS1 was dominated by *Tepidicaulis* (21.29%) *Hyphobacterium* (8.36%) and Thalassospira (7.745%). HS2 shares dominance by AB015524_f_uc (from order Rhodothermales) (20.70%), JF272039_g (from family Phycisphaeraceae) (9.82%) and *Hyphobacterium* (9.02%). In addition to *Thiomicrospira* (50.58%), HS3 was co-dominated by *Sulfurimonas* (9.77%) and AF170421_g (from order Chromatiales) (8.0699%).

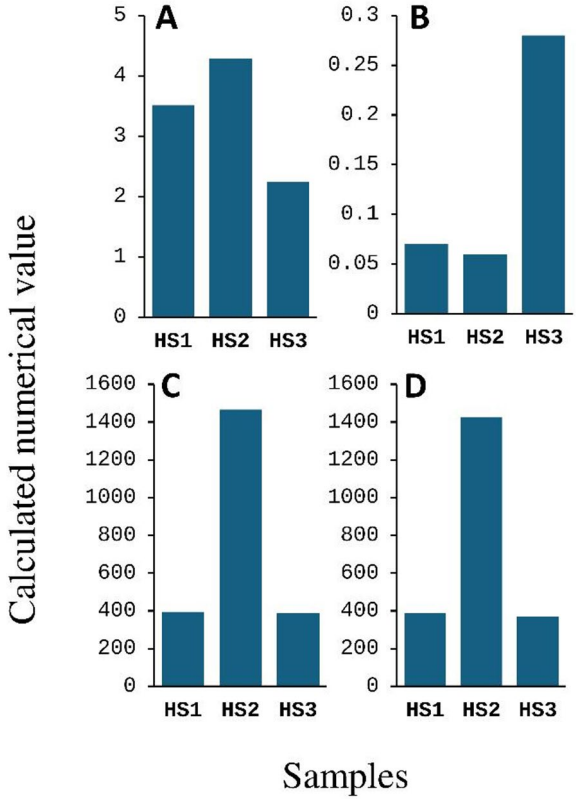

Fig. 2. Alpha diversity indices illustrating bacterial richness and evenness across the identified bacteriomes: (**A**) Shannon, (**B**) Simpson, (**C**) ACE, and (**D**) Chao. For all indices, higher values indicate greater diversity, except for the Simpson index, where higher values correspond to lower diversity.

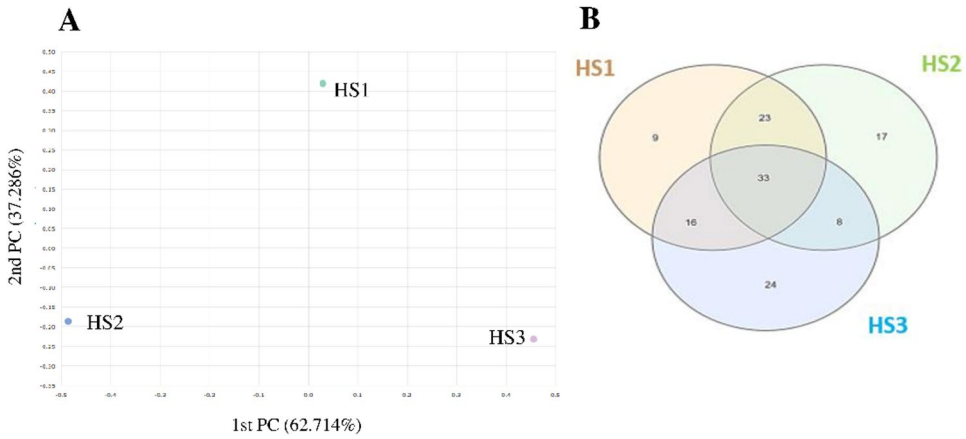

Fig. 3. PCoA plot showing the clustering patterns of the three bacteriomes (**A**), and a Venn diagram illustrating the distribution of shared and unique OTUs among them (**B**).

Identification of genera with potential bioremediation capabilities

Based on previous reports, this study identified several bacterial genera with significant biodegradation potential (Table 2). These taxa include bacteria capable of degrading polycyclic aromatic hydrocarbons (PAHs) (e.g., *Pseudomonas*, *Acidovorax*), organohalide-respiring bacteria (OHRB) (e.g., *Pseudomonas* and *Desulfuromonas*), and metal-reducing bacteria (e.g., *Desulfovibrio*, *Shewanella*). Additionally, genera such as *Pseudomonas*, *Acinetobacter*, and *Alcanivorax* exhibited strong potential for oil and hydrocarbon degradation.

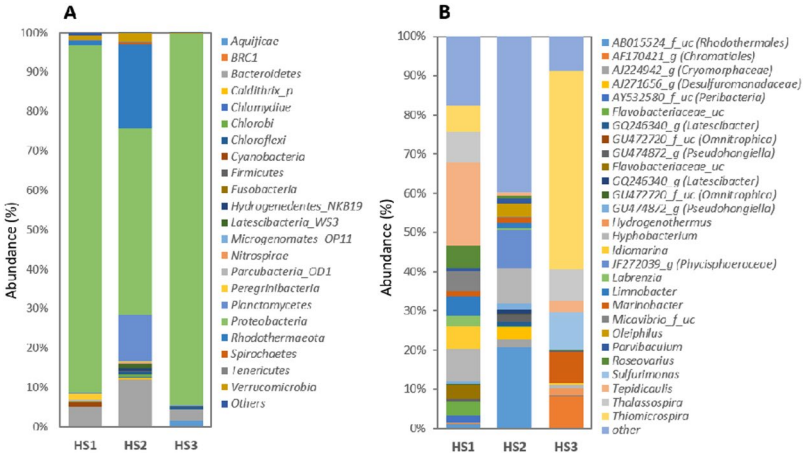

Fig. 4. Bacterial community composition at the (**A**) phylum and (**B**) genus levels.

Genus	Pollutants	HS1	HS2	HS3	References
Pseudomonas	Crude oil, Polyethylene, Heavy oil, PAHs, OHRB	+	+	+	37–41
Acidovorax	PAHs, BTEX	+	-	-	42,43
Acinetobacter	Phenol, Petroleum Hydrocarbons	+	-	-	44,45
Alcanivorax	Beyond oil degradation, Long chain alkanes, Petroleum hydrocarbon	+	+	-	46–48
Anoxybacillus	Azo dye	+	-	-	49
Arenibacter	PAHs	-	+	-	50
Bacillus	Petroleum Hydrocarbon	-	+	+	51
Defluviimonas	PAHs	-	+	+	52
Desulfovibrio	U(VI) reduction	-	+	-	53
Desulfuromonas	OHRB, Fe (III), Mn, U (VI) reduction	+	+	-	54–57
Marinobacter	Hydrocarbons	+	+	+	58
Nitrosomonas	Quinoline, Crude oil	-	+	-	59,60
Shewanella	Iron, Mn, U (VI) reducing bacteria, Sulfonamides, PAHs	-	+	-	54,61–63
Sulfurimonas	Alkanes, PAHs, Nitrate-Reduction	-	-	+	64,65

Table 2. Potential xenobiotic degrading genera identified in this study.

Functional biomarkers for bioremediation prediction

PICRUSt analysis predicted the functional potential of the studied bacteriomes, indicating the presence of genes implicated in key enzymatic steps of biodegradation pathways. The red-labeled enzymes detailed in this metabolic map represent crucial functional biomarkers that directly relate to the bioremediation potential of the detected bacterial populations (Fig. 5). The functional analysis identified 13 highly significant predicted genes that serve as crucial functional biomarkers within the bacteriome. These genes are organized into four major catabolic pathways that ultimately feed into the TCA cycle. Specifically, the Catechol Bioremediation Pathway is characterized by genes for catechol 2,3-dioxygenase and phenol monooxygenase. The Benzoate Pathway involves genes encoding naphthalene dioxygenase and vanillate monooxygenase. The Glyoxylate & Dicarboxylate Metabolism pathway includes genes for haloacetate dehalogenase and biphenyl 2,3-dioxygenase. Finally, the Xylene Bioremediation Pathway is distinctively marked by the presence of the phenol monooxygenase gene. The provided metabolic map illustrates these pathways highlights a fundamental principle of microbial catabolism. Initial activation steps, catalyzed by key enzymes like Vanillate monooxygenase ferredoxin subunit, Biphenyl 2,3-dioxygenase beta subunit, and Naphthalene 1,2-dioxygenase subunit beta, facilitate the initial transformation of structurally diverse contaminants (e.g., biphenyl, naphthalene, vanillate, and chloroacetate). The products are then efficiently directed toward key central intermediates, primarily Catechol and Protocatechuate, catalyzed by enzymes such as Phenol 2-monooxygenase and Protocatechuate 3,4-dioxygenase subunit beta. This metabolic funnel ultimately directs chemical energy into the TCA cycle via the Benzoate Biodegradation and Glyoxylate & Dicarboxylate pathways. The identification of these predicted genes provides an indication of the potential metabolic capabilities of the bacteriomes based on taxonomic inference. Further studies may help to validate and refine the in situ activity and ecological relevance of these pathways, for example through shotgun metagenomics, transcriptomics, or cultivation-based approaches.

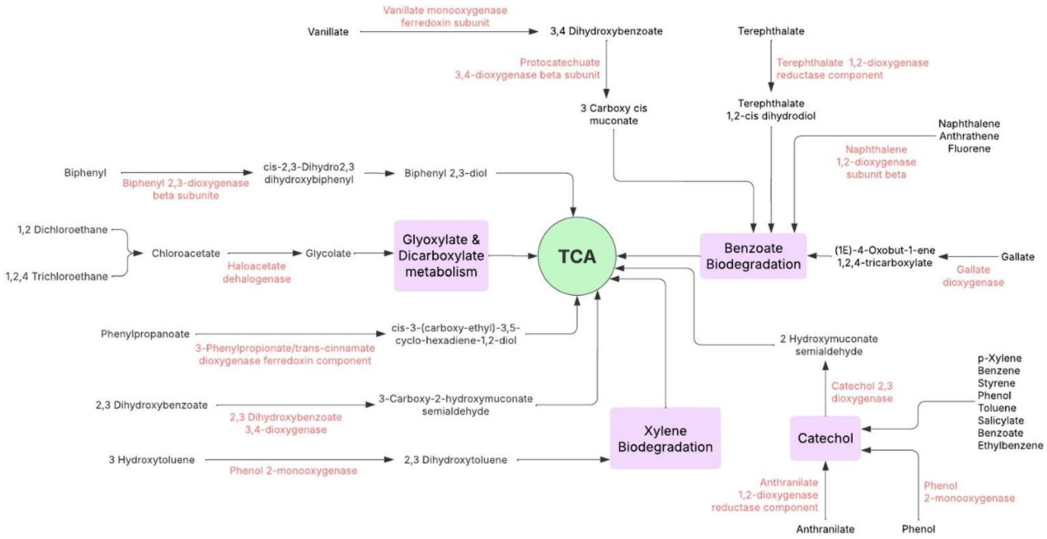

Fig. 5. Xenobiotic degradation pathways proposed in this study. Functional biomarker genes predicted and detected using PICRUSt tool in this study are marked in red. Pathways were adapted from KEGG35. (permission number 254109).

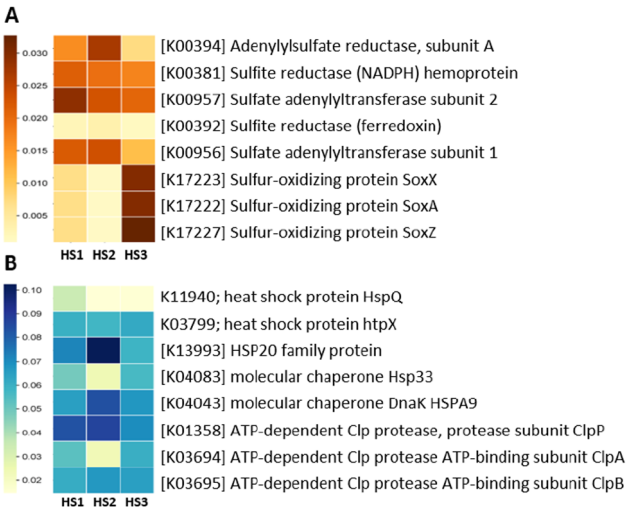

Fig. 6. Heatmaps illustrating functional biomarkers predicted using PICRUSt: (**A**) sulfur cycle-related and (**B**) heat shock response-related biomarkers.

Prediction of metabolic functions related to the sulfur cycle and heat stress response

Due to the inherent sulfur-rich biogeochemistry of the sampling sites, particular emphasis was placed on analyzing functional biomarkers within the predicted bacteriome related to the sulfur cycle. This targeted investigation provides a direct link between the detected microbial taxa and the specific enzymatic machinery required to mediate the biogeochemical cycling of sulfur at the site, which is vital for understanding environmental health and potential remediation strategies. The heatmap analysis of PICRUSt-predicted genes for the sulfur cycle (Fig. 6A) reveals distinct specializations across the three communities. HS2 exhibits the highest genetic potential for dissimilatory sulfate reduction (DSR) (genes K00394 and K00392, involved in energy production), justifying its potential to thrive in anaerobic conditions by reducing sulfur compounds. Conversely, HS1 demonstrates the highest potential for assimilatory sulfate reduction (ASR) (genes K00381, K00957, K00956, necessary for biomass production), suggesting a focus on growth and sulfur incorporation into essential biomolecules. Furthermore, both HS1 and HS2 show a significantly higher genetic capacity for sulfide oxidation (SoxX, SoxA, and SoxZ genes) compared to HS3, justifying their ability to efficiently recycle reduced sulfur compounds and maintain sulfur redox homeostasis in their respective environments. In addition to the sulfur-rich characteristics of the sampling sites, special attention was given to identifying functional biomarkers and genes linked to the heat shock response considering the overall heat- and stress-associated nature of the sampling sites in this study. A broad range of heat-responsive genes was detected across the sampled sites. The distribution of these genes

varied notably among the three sites (Fig. 6B). HspQ exhibited the highest abundance in HS1, followed by HS3 and HS2. Similarly, Hsp33 showed its highest level in HS1, with higher levels in HS3 than in HS2. Conversely, HSP20 is most represented in HS2, while DnaK is also highest in HS2. The protein htpX is most abundant in HS3. The protease, ClpP is most represented in HS2, while ClpA has its highest value in HS3, and ClpB is most abundant in HS2.

Phylogenetic analysis and ecological interpretation

Figure (7) illustrates the phylogenetic relationships among the bacterial genera identified in this study that are potentially involved in xenobiotic degradation (Table 2), sulfur cycling, and thermophily (Table 3). based on a Neighbor-Joining tree constructed from 16 S rRNA gene sequences. The phylogenetic structure and functional profiling confirm that the analyzed samples harbor a diverse yet specialized community, optimized for biogeochemical sulfur cycling and adapted to thermophilic conditions, while simultaneously retaining high bioremediation potential. The tree is annotated to highlight three critical functional characteristics for each

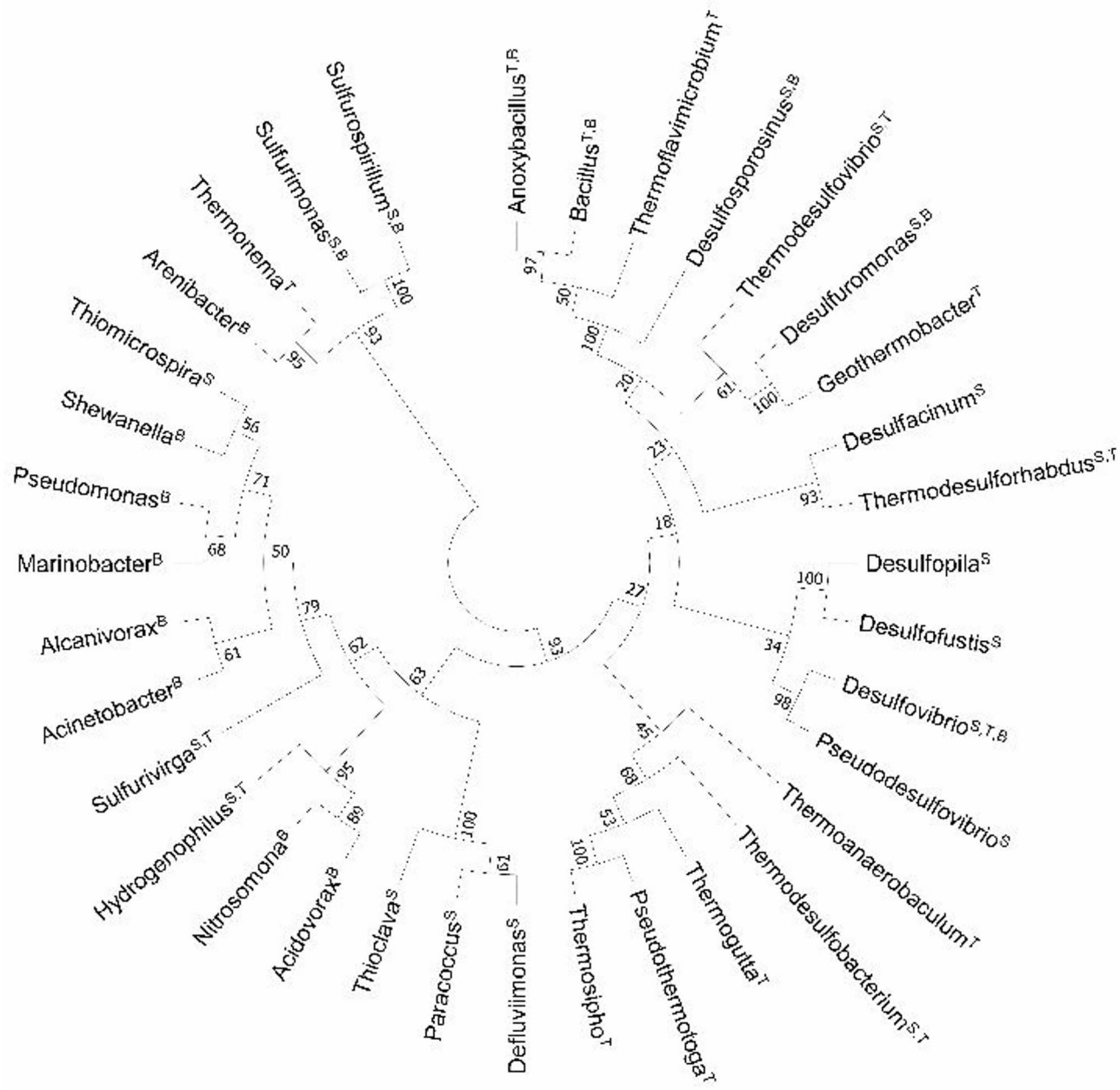

Fig. 7. Neighbor-joining circular phylogenetic tree based on 16 S rRNA gene sequences, illustrating bacterial genera identified in this study that are potentially involved in xenobiotic biodegradation, sulfur cycling, and thermophily. Superscript letters following each genus indicate functional traits: (S) sulfur-metabolizing, (B) bioremediation-related, and (T) thermophilic genera. Multiple designations (e.g., S–B or S–T) reflect multifunctional roles in sulfur metabolism, thermophily, and bioremediation.

Genus	HS1	HS2	HS3	References
Sulfur bacteria				
Desulfacinum	-	+	+	[66]
Desulfofustis	+	+	-	[67]
Desulfopila	-	+	-	[68]
Desulfosporosinus	-	+	-	[69]
Desulfovibrio	-	+	-	[70]
Desulfuromonas	-	+	-	[71]
Thioalkalispira	+	-	+	[72]
Thioclava	+	+		[73]
Thermodesulforhabdus	-	-	+	[74]
Sulfurospirillum	+	-	-	[75]
Sulfurimonas	-	-	+	[76]
Sulfurivirga	+	-	+	[77]
Thiomicrospira	+	-	+	[78]
Paracoccus	+	+	+	[79]
Pseudodesulfovibrio	+	+	-	[80]
Thermophilic bacteria				
Anoxybacillus	+	-	-	[81]
Desulfacinum	-	+	+	[82]
Geothermobacter	-	+		[83]
Pseudothermotoga	+	-	-	[84]
Thermoanaerobaculum	-	-	+	[85]
Thermodesulfobacterium	+	-	-	[86]
Thermodesulforhabdus	-	-	+	[87]
Thermodesulfovibri	-	+	-	[88]
Thermoflavimicrobium	-	+	-	[89]
Thermogutta	+	-	-	[90]
Thermonema	-	-	+	[91]
Thermosipho	+	-	+	[92]
Hydrogenophilus	+	-	+	[93]
Sulfurivirga	+	-	+	[94]

Table 3. Potential sulfur-cycle–associated and thermophilic bacterial genera identified in this study.

genus: (S) for sulfur metabolism (oxidation/reduction), (B) for bioremediation-related potential, and (T) for thermophilic adaptation. The distribution of functional characteristics across the tree suggests a community highly adapted to sulfur cycling and elevated temperatures, while also possessing significant potential for remediation. A large, strongly supported clade is characterized by genera possessing sulfur-metabolizing and thermophilic traits. This group includes several sulfate-reducing bacteria (SRB) like *Desulfovibrio*, *Thermodesulfovibrio*, and *Thermodesulforhabdus*, indicating that both sulfur reduction and oxidation are major processes in the analyzed samples. A separate, well-supported cluster comprises genera, primarily known for their roles in bioremediation. This includes genera like *Acinetobacter*, *Marinobacter*, and *Pseudomonas*, which are widely studied for their ability to degrade various organic pollutants. The presence of these genera suggests the bacteriome is resilient and capable of bioremediating diverse compounds. The most ecologically significant observation is the presence of several genera exhibiting overlapping functions. *Desulfovibrio* is uniquely categorized as S, T, and B, signifying its critical and versatile role as a high-temperature sulfate-reducer with potential applications in pollutant remediation. Similarly, *Sulfurimonas* (S, B) and *Sulfurospirillum* (S, B) couple sulfur cycling with bioremediation capabilities, highlighting the interconnected nature of biogeochemical processes within this microbial consortium.

Discussion

Pharaoh's Bath (Hammam Pharaon), located about 250 km from Cairo, comprises natural sulfur-rich hot springs flowing into a 100-m lake by the sea and is well known for therapeutic tourism[95]. However, its microbiological profile remains largely unexplored. This study investigated the bacteriomes of three sites from these hot springs, focusing on genera associated with bioremediation and key functional biomarkers of these processes.

Thermally enhanced bioremediation is a novel concept that explores the combination of thermal treatment and bioremediation to enhance the low efficiency and long duration of bioremediation. The thermal effects on the physical, chemical and biological characteristics of soil, and contaminants bioavailability and bacterial commuity metabolic activities. Specifically, the increase in temperature within a suitable range can proliferate enzymes enrichment, extracellular polysaccharides and biosurfactants production, and further improving

bioremediation efficacy[96]. Microorganisms that thrive at temperatures from 70 °C to 80 °C are classified as extreme thermophiles[97]. Moreover, a sufficient increase in temperature can enhance bioremediation. Yadav et al. (2012)[98] recorded that the bioremediation of toluene was increased two folds for every 10 °C rise in soil temperature.

Proteobacteria dominated all samples, followed by Bacteroidetes in HS1 and HS3 and Rhodothermaeota in HS2. While this agrees with previous studies, the second most abundant phylum varied. Sharma et al. (2020)[99] reported Proteobacteria and Firmicutes in Sikkim hot springs, and DeCastro et al. (2021)[100] reported Proteobacteria and Deinococcus-Thermus in Spain. Similar dominance of Proteobacteria has been observed in the Himalayas[101] and Kharasinpur[102], whereas other sites showed different patterns, such as Actinobacteria in Rajgir, India[103] and Firmicutes in Jizan, Saudi Arabia[104].

The predominant genera varied across the three bacteriomes. HS1 was dominated by *Tepidicaulis*, *Hyphobacterium*, and *Thalassospira*; HS2 by AB015524_f_uc (Rhodothermales), JF272039_g (Phycisphaeraceae), and *Hyphobacterium*; and HS3 by *Thiomicrospira*, *Sulfurimonas*, and AF170421_g (Chromatiales). These findings align with previous studies highlighting hot spring microbial diversity, such as the abundance of *Exiguobacterium* and *Pseudomonas* in Polok and *Thermodesulfovibrio* and *Sulfurihydrogenibium* in MDV[89,90]. Similar variability has been reported in Pharaoh's Bath, where different studies identified genera such as *Geobacillus*, *Rhodothermus*, *Thermus*[105], *Bacillus*, *Anoxybacillus*, and *Geobacillus*[106] using conventional and molecular methods.

16 S rRNA gene analysis of the three sites identified microbial taxa with potential degradative functions (Tables 2 and 3). Proteobacteria, commonly dominant in hydrocarbon-contaminated environments, plays a key role in biodegradation[107], alongside Actinobacteria, due to their specialized degradative enzymes. The dominance of Proteobacteria in these samples therefore indicates a strong potential for hydrocarbon biodegradation.

Functional prediction using PICRUSt suggested 13 key genes linked to major biodegradation pathways, supporting a strong degradation potential in the studied bacteriomes. These suggested genes are associated with the breakdown of a wide range of pollutants, including aromatic hydrocarbons, phenols, halogenated compounds, pharmaceuticals, and plastic-derived substances[108–113], supporting a strong biodegradation potential in the studied bacteriomes. Similar findings were reported by Saxena et al. (2017)[18], who identified abundant hydrocarbon degradation genes in Anhoni hot springs, dominated by *Pseudomonas stutzeri* and *Acidovorax* sp. Additionally, Saeed et al. (2020)[114] demonstrated molybdate bioremediation in Pharaoh's Bath, further highlighting its environmental remediation potential of the studied site[115].

In addition to hydrocarbon degradation, sulfur metabolism pathways were also predicted. PICRUSt analysis identified genes associated with both DSR and ASR pathways. In DSR, the aprA gene (K00394) involved in APS-to-sulfite conversion was detected, although additional genes are required for complete sulfate reduction[116,117]. In ASR, key genes including cysN (K00956), cysD (K00957), cysI (K00381), and sir (K00392) were identified, supporting partial pathway functionality. The presence of SOX genes further indicates thiosulfate oxidation potential, relevant for treating sulfur-rich industrial wastewater[118–121]. Similar sulfur metabolism genes have been reported in hot springs from Taiwan[122] and New Zealand[123]. Together, these findings highlight an expanded bioremediation potential through sulfur cycling, complementing previously identified hydrocarbon degradation capabilities.

PICRUSt analysis also predicted a diverse set of heat shock protein genes, indicating strong microbial adaptation to environmental stress. These proteins support cellular stability by maintaining protein folding, preventing aggregation, and facilitating repair under extreme conditions. Key HSPs such as DnaK, ClpP, Hsp33, and HSP20 are associated with tolerance to thermal, oxidative, and chemical stress[124–129], reflecting active stress response mechanisms within the bacteriomes.

Although Archaea, eukaryotes, and viruses occur in hot spring environments, numerous studies show that bacteria dominate both community abundance and sulfur-related functional potential in many systems[130,131]. In some surveys, bacteria account for up to 95% or more of total microbial sequences, while Archaea represent only a small fraction[132]. Eukaryotes are also minor contributors, comprising about 1.3% of taxa in hot spring biofilms. Viruses are present, but their ecological roles especially in sulfur cycling remain less well characterized[133].

Bacteriome profiling in this study was conducted using 16 S rRNA gene amplicon sequencing, a widely applied and cost-effective approach for characterizing microbial diversity. Although shotgun metagenomics provides higher taxonomic resolution and direct functional insights, its greater cost and computational demands often limit its overall use[134]. While this method provides a rapid overview of community composition, it is inherently limited by primer bias, variation in gene copy number, and reduced taxonomic resolution at the species level[135,136]. Moreover, functional predictions inferred using PICRUSt are based on taxonomic profiles and should therefore be considered indicative rather than confirmatory.

Future work should focus on integrating high-resolution physicochemical characterization (e.g., salinity, pH, metal concentrations) with multi-omics approaches to strengthen ecological interpretation. Specifically, shotgun metagenomics targeting key functional genes involved in pollutants degradation will provide direct evidence of metabolic capacity. In addition, enrichment and isolation of key thermophilic taxa will enable functional validation under controlled laboratory conditions. Finally, in situ or pilot-scale bioreactor experiments using industrial effluents are recommended to evaluate the practical applicability and stability of these microbial communities under real-world conditions. Such approaches will be essential to confirm the functional potential inferred in this study and to bridge the gap between prediction and application.

Conclusion

This study offers an overview of the taxonomic composition and inferred metabolic potential of thermophilic bacteriomes inhabiting Pharaoh's Bath Hot Springs in South Sinai, Egypt. The results reveal distinct community structures among the three sites, likely shaped by temperature gradients and associated geochemical conditions, reflecting strong environmental selection pressures. Predictive functional analyses indicate the possible presence

of genes putatively involved in sulfur cycling, stress response, and xenobiotic degradation. Collectively, the hot springs studied represent promising reservoirs of bacterial communities with potential biotechnological and bioremediation applications. These PICRUSt-based functional insights should be interpreted with caution, as they do not reflect direct measurements of gene expression or activity, but rather indicate potential trends. Therefore, validation using complementary approaches such as shotgun metagenomics, metatranscriptomics, and targeted assays is required. Future work should integrate physicochemical characterization with multi-omics analyses targeting key sulfur and biodegradation related genes, alongside enrichment of key taxa and bioreactor-based experiments to confirm functional potential under environmentally relevant and application-relevant conditions.

Data availability

Raw sequence data from this research have been submitted to the NCBI database and can be accessed via the BioProject accession number PRJNA1354486.

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Author contributions

W.S.E. designed the study, collected samples and managed the sequencing process. M.I., A.M.S and S.A.D performed alpha and beta diversity analysis. M.I., S.A.D., A.M.S. and W.S.E. provided input for data interpretation. M.I., S.A.D., A.M.S. and W.S.E. drafted the manuscript, W.S.E. revised the manuscript. All authors read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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