



# Metagenomic Analysis of Bacterial Diversity in an Acidic Sulfur Mud Pool in Kragilan, Banten Using Next-Generation Sequencing

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**Abstract.** The exploration of microorganisms from extreme environments in Indonesia remains limited, despite the country's richness in diverse and unique ecosystems. This study aimed to investigate the bacterial community diversity in a highly acidic sulfur mud pool in Kragilan, Banten, using the Oxford Nanopore Technologies Next-Generation Sequencing (NGS) platform. The mud sample was analyzed for its taxonomic composition and physiological characteristics based on literature. In situ measurements revealed a temperature of 28°C and a pH of 1.5. Metagenomic analysis identified a total of 157 bacterial genera and 412 species. The most dominant genus was *Bacillus* (31.01%), followed by *Paenibacillus* (19.42%) and *Staphylococcus* (16.53%). At the species level, *Bacillus cereus* (20.88%) showed the highest abundance. Despite the highly acidic environment, physiological analysis indicated that the majority of the identified bacteria were neutrophilic (53.70%) and mesophilic (75.67%), suggesting the presence of robust adaptation mechanisms to acidic conditions. Furthermore, several acidophilic species such as *Acidibrevibacterium fodinaquatile*, *Alicyclobacillus disulfidooxidans*, *Acidicaldus organivorans*, and *Alicyclobacillus acidiphilus* were also detected. These findings offer important preliminary insights into the complexity of bacterial communities inhabiting underexplored acidic environments in Indonesia and highlight the critical need for further research in such unique ecological niches. The exploration of acidic habitats holds significant potential for the discovery of novel acidophilic bacterial candidates with valuable industrial biotechnological applications, including

industrial biotechnological applications, including biomining, the production of pH-stable enzymes, and environmental bioremediation.

**Keywords:** Acidophile, Bacterial Diversity, Metagenomics, Next-Generation Sequencing, Sulfur Mud Pool.

## 1 Introduction

Extremophiles, particularly acidophiles, are microorganisms that thrive in low-pH or highly acidic environments. To survive under such harsh conditions, they have developed sophisticated cellular adaptation mechanisms, such as acid-resistant cell membranes and proton-pumping systems that help maintain a neutral intracellular pH despite the acidic surroundings [1]. Acidophilic bacteria are known to produce acid-stable enzymes like proteases, xylanases, and  $\alpha$ -amylases, making them highly valuable for various industrial applications including food processing, biofuel production, pulp and paper manufacturing, and bioremediation. Additionally, these microorganisms play a crucial role in biomining, an eco-friendly technique for extracting metals like copper, gold, nickel, and uranium from low-grade ores [2–8].

Metagenomic analysis is crucial for exploring the microorganisms present in extreme environments, thereby maximizing the potential of these microorganisms for biotechnological applications [9]. Several studies have investigated bacterial communities in extreme environments to assess their potential [10–13]. Metagenomic analysis can analyze the presence of complex microbes in a given environment without the need to culture the bacteria in the laboratory. With metagenomic analysis, researchers can identify the taxonomic diversity of microbial communities in environments such as soil, water, and living organisms, thereby exploring these microbes' potential [14–16].

In Indonesia, research on acidophilic bacteria is lack of study, particularly from naturally acidic environments such as sulfurous rivers and geothermal hot springs. Few example of explored acidophilic bacteria from Indonesia are *Acidithiobacillus sp.* KL3 that isolated from sulfur-rich river sediment [8], and diverse acidophilic microbial communities that identified in geothermal springs in the Kamojang area, West Java [17]. These findings are the prove that research about acidophiles has begun to emerge, commonly inhabit extreme environments such as sulfur springs and volcanic geothermal systems in Indonesia. The research mostly done by doing 16s rRNA methods. This method only offer single bacteria analysis whereas bacterial communities should be exist within sample.

The advancement of Next-Generation Sequencing (NGS) technologies, particularly Oxford Nanopore sequencing, has revolutionized the exploration of microbial communities by enabling direct sequencing of environmental DNA without the need for prior culturing or laboratory isolation. This culture-independent approach allows for rapid and high-resolution taxonomic profiling as well as detection of functional genes from complex microbial communities [18–20]. In this preliminary study, we employed the Oxford Nanopore Technologies platform to explore the diversity of bacterial genera and species in sulfur mud sample collected from Kragilan, Banten, Indonesia. The findings are expected to contribute to a broader understanding of microbial diversity in

acidic environments and serve as a foundation for future in-depth exploration in similar remote ecosystems for applications in industrial biotechnology.

## 2 Materials and Methods

### 2.1 Sample Collection

A sulfuric mud sample was collected from the center of a natural sulfur mud pool in Kragilan, Banten, Indonesia, in the early morning of November 2024. The sample was placed into a sterile container, immediately sealed, and transported in an ice box maintained at 4 °C. In the laboratory, the sample was filtered using sterile filter paper under aseptic conditions. The filter paper containing the retained biomass was then frozen at -40 °C.

### 2.2 Metagenomic Analysis

The frozen filter paper was subsequently sent to PT Genetika Science for metagenomic analysis using the Oxford Nanopore Technologies next-generation sequencing platform. The DNA from the sample were extracted and sequenced by Oxford Nanopore Technologies. Samples were sequenced between 15 minutes until 24 hours depending on the GridION X5 Mk1. The sequence then were read using guppy (v6.1.5).

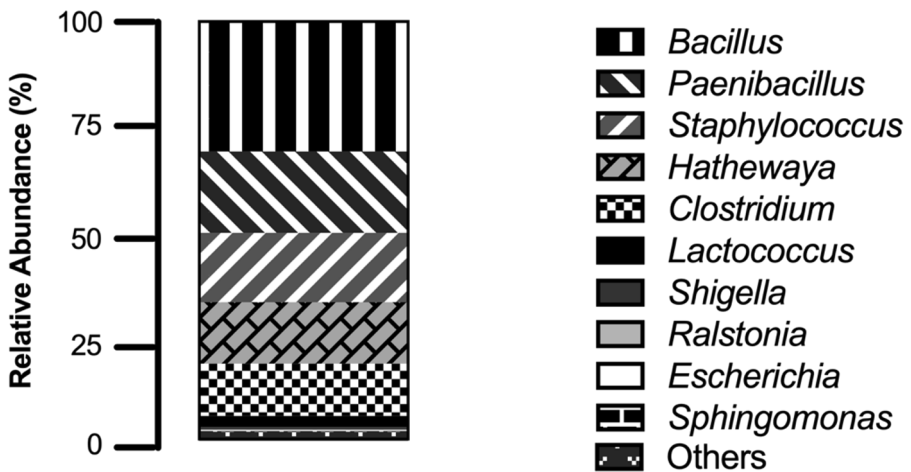
## 3 Results

In situ measurements of temperature and pH were conducted at the sulfur mud pool site using a calibrated digital thermometer and portable pH meter. The results showed that the water temperature was 28 °C, while the pH value was measured at 1.5. The measurements and sampling were taken during clear weather conditions without rainfall to ensure accuracy and stability of the readings. The location is shown in Figure 1.



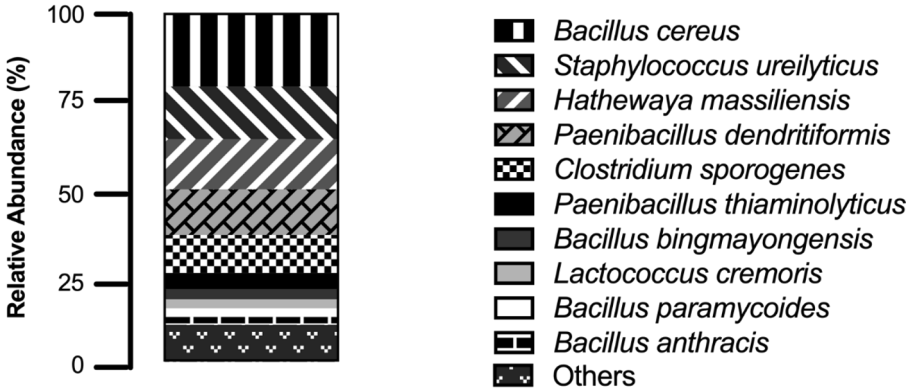
**Fig. 1.** Location of the sulfur mud pool in Kragilan, Banten.

Next-generation sequencing (NGS) analysis of the sample from the sulfur mud pool revealed a high level of bacterial biodiversity. Using the Oxford Nanopore Technologies platform, a total of 157 bacterial genera and 412 species were identified, comprising 43,747 individual bacterial reads. Among the detected genera, *Bacillus* was the most dominant, with a relative abundance of 31.01%, corresponding to 13,564 individual reads, as shown in Figure 2. Other frequently identified genera, in descending order of abundance, included *Paenibacillus* (19.42%), *Staphylococcus* (16.53%), *Hathewayia* (14.66%), *Clostridium* (12.48%), *Lactococcus* (2.63%), *Shigella* (0.60%), *Ralstonia* (0.23%), *Escherichia* (0.16%), and *Sphingomonas* (0.15%). The others category accounted for 2.14% of the total, representing genera outside the top 10 most abundant.



**Fig. 2.** Relative abundance of the top 10 bacterial genera identified from sulfur mud pool in Kragilan, Banten of the sulfur mud pool in Kragilan, Banten.

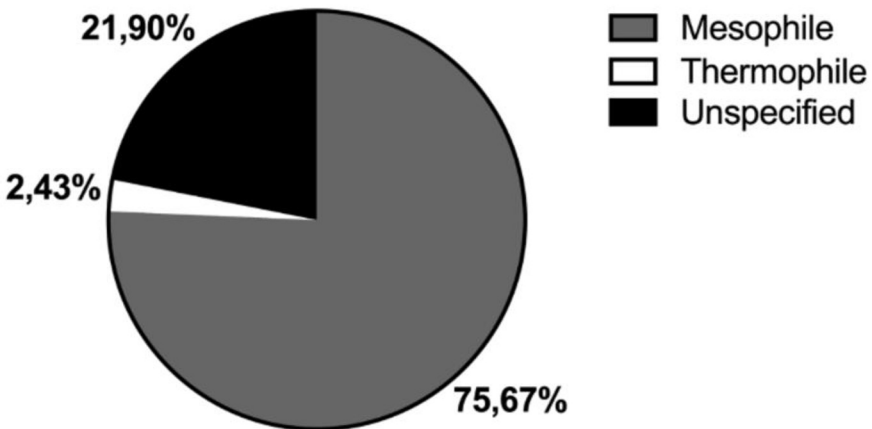
At the species level, *Bacillus cereus* was the most dominant, with a relative abundance of 20.88% and a total of 9,134 individual reads, as presented in Figure 3. Other predominant species included *Staphylococcus ureilyticus* (15.12%), *Hathewayia masilensis* (14.43%), *Paenibacillus dendritiformis* (13.10%), *Clostridium sporogenes* (10.93%), *Paenibacillus thiaminolyticus* (10.93%), *Bacillus bingmayongensis* (4.70%), *Lactococcus cremoris* (2.85%), *Bacillus paramycoides* (2.61%), and *Bacillus anthracis* (2.50%). The remaining bacterial species, grouped under the others category, accounted for 10.64% of the total relative abundance.



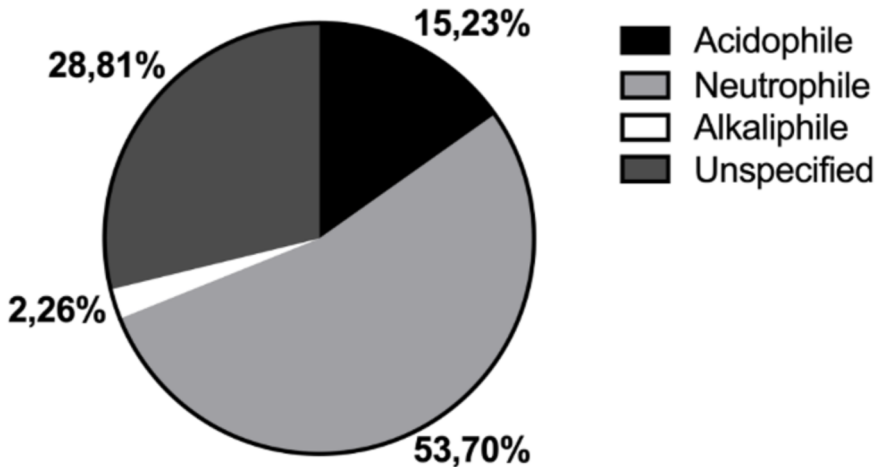
**Fig. 3.** Relative abundance of the top 10 bacterial species identified from sulfur mud pool in Kragilan, Banten.

Physiological characterization of the identified bacteria was performed based on literature review of the growth temperature and pH ranges for each species. Based on temperature classification (Figure 4A), the majority of the identified species were mesophilic (20–45°C), comprising 75.67% of the total. A smaller proportion were classified as thermophilic (45–80°C), representing 2.43%, while the remaining 21.90% were categorized as unspecified due to lack of temperature growth data in the literature. A similar analysis was conducted for pH growth range classification (Figure 4B). The results indicated that most species were neutrophilic (pH 5.5–8), accounting for 53.70%, followed by acidophilic species (pH <5.5) at 15.23%, and alkaliphilic species (pH >8) at 2.26%. The remaining species were assigned to the unspecified category due to the unavailability of pH growth range information in the referenced literature.

**(A)**



(B)



**Fig. 4.** Physiological characterization of the identified bacteria based on their adaptability to (A) temperature range and (B) pH range.

## 4 Discussion

The exploration of bacterial biodiversity from a sulfur mud pool in Kragilan, Banten, revealed that the sample temperature was measured at 28 °C. This temperature is noticeably lower than that of many other sulfur mud pool in Indonesia, which can exceed 50 °C [21], suggesting the absence of intense geothermal activity at the site [22]. However, the measured pH value was extremely acidic, at 1.5, which contradicts common public perception that sulfur mud baths are suitable for alternative dermatological therapy. In fact, such highly acidic conditions may pose risks of skin irritation and tissue damage [23]. Next-generation sequencing (NGS) analysis revealed a highly diverse bacterial community, with members belonging to various genera. Among these, the most dominant genus was *Bacillus* (Figure 2), with *Bacillus cereus* being the most abundant species (Figure 3). Like other species in the *Bacillus* genus, *B. cereus* is commonly found in soil due to its low nutritional requirements for growth. Although it is not acidophile, *B. cereus* is classified as a neutrophile and mesophile bacterium and is recognized as a potential human pathogen. It can produce cereulide, a toxin known to be stable under extreme pH conditions [24, 25]. Physiological characterization based on literature review indicated that most of the identified bacteria were mesophilic (Figure 4A), consistent with the moderate temperature measured at the site. Interestingly, despite the highly acidic environment, the majority of bacterial species were classified as neutrophile (Figure 4B), suggesting the presence of unique adaptation mechanisms that enable their survival in low-pH environments [26]. Notably, several acidophilic species were also identified in the sample, including *Acidibrevibacterium fodinaquatile*

[27], *Alicyclobacillus disulfidooxidans* [28], *Acidocaldus organivorans* [29], and *Alicyclobacillus acidiphilus* [30], highlighting the potential for further exploration of acidophilic bacteria and their biotechnological applications [3, 31, 32]. Furthermore, the analysis identified several bacterial species that exhibit tolerance to acidic environments, including *Shigella spp.* [33], *Salmonella enterica* [34], *Clostridium aciditolerans* [35], *Clostridium drakei* [36], *Paenibacillus sp.* [37], *Staphylococcus aureus* [38], and *Klebsiella pneumoniae* [39]. Acidophilic bacteria hold significant industrial potential, particularly in the field of biotechnology. They are widely utilized in biomining applications, where microbes facilitate the extraction of base metals such as copper and nickel, as well as precious metals like gold and uranium, from low-grade ores [2]. These bioprocesses offer advantages including lower energy requirements and environmental sustainability [5]. Additionally, enzymes derived from acidophilic bacteria exhibit remarkable stability under low pH and high temperature conditions, making them highly valuable in industries such as starch processing, fruit juice production, animal feed manufacturing, and baking [5]. Acidophiles also play a crucial role in the bioremediation of environments contaminated with acid mine drainage or heavy metals [40]. This study offers a preliminary insight into the diversity and potential of acidophilic bacteria in the sulfur mud pool in Kragilan, Banten. Further research is encouraged to isolate and characterize these microbes, laying the groundwork for their potential industrial applications in sustainable bioprocesses.

## 5 Conclusion

This study successfully revealed a high bacterial diversity in the Kragilan sulfur mud pool, which has a highly acidic condition (pH 1.5). Although dominated by neutrophile bacteria such as *Bacillus cereus*, the presence of several acidophilic and other acid-tolerant bacterial species indicates the existence of unique and effective adaptation mechanisms for survival in extreme environments. This finding confirms that the site is a promising resource for bioprospecting new microorganisms with the potential for producing acid-stable enzymes and other biotechnological applications, such as in biomining and bioremediation. Further research, particularly the isolation and functional characterization of the identified microbes, is highly recommended to fully harness their potential in sustainable bioprocess industries.

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**Disclosure of Interests.** The authors declare no conflicts of interest.

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