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Isolation and Characterization of Thermophilic Bacteria from Ikogosi Warm Spring, Ekiti State, Nigeria

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ABSTRACT

Thermophilic microorganisms are adapted to thrive at high temperatures and are sources of thermostable enzymes with several potential industrial applications. The aim of this study was to isolate, characterize, and identify thermophilic bacteria from Ikogosi warm spring in Ekiti state, Nigeria. Water, sand, and rock mattings samples were obtained from different sites of the spring in sterilized vials and transported in an ice chest to the laboratory for analysis. Then, 1.5 mL of water and 1 g of each sediment were cultured on compounded Castenholz Tryptone-Yeast Extract medium at 70°C for 96 h for bacteria isolation. The study yielded twenty-seven thermophilic bacteria, which were identified based on morphological and biochemical characteristics. Results showed that out of the 27 isolates, 15% were Gram-negative organisms, and 85% were Gram-positive organisms. Based on morphological and various biochemical characteristics, the 27 isolates were closely related to the genera *Thermus* and *Geobacillus.* Further studies will screen the isolated bacteria for their ability to produce various enzymes.

Keywords: Castenholz TYE medium, *Geobacillus* spp., Geothermal springs, Ikogosi, Thermophilic bacteria.

Introduction

The microbial world remains the largest unexplored biodiversity reservoir on Earth and has been a source of various species, products, and processes of biotechnological importance.[1] Microorganisms have evolved to exploit a wide range of energy sources and survive in virtually every habitat.[2] The potential for new microbial industrial applications is as diverse as the variety of environments in which they exist.[3] It has been speculated that thermophilic bacteria were among the first organisms during the primordial birthing of the earth, which was characterized by extremely high temperature and, as a result, are adapted to thrive at such temperatures.[4] Thermophilic microorganisms occur widely in both naturally occurring and anthropogenic high-temperature environments such as geothermal springs, hydrothermal vents, hot taps, thermally contaminated waters, and decaying matters.[5]

Interest in the isolation of thermophilic bacteria was spurred by their tremendous potential as sources of thermostable biomolecules with diverse applications in biotechnological processes. The most significant discovery has been the isolation of *Thermus aquaticus*, which was first isolated from the Grand Prismatic Spring in the United States Yellowstone National Park by Thomas D. Brock in 1969 and has since been isolated from thermal habitats across the world.[6] A thermostable DNA polymerase, Taq polymerase, extracted from *Thermus aquaticus* proved decades later to be crucial in polymerase chain reaction (PCR) and in effect has made significant contributions to molecular biology, DNA fingerprinting, forensic analysis, agriculture, and disease diagnostics. Various species of thermophilic bacteria have been isolated from hot springs in China,[7] Ethiopia,[8] Jordan,[9] Malaysia,[10] Saudi Arabia,[11] and India.[12]

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The Ikogosi warm spring, Ekiti state has been characterized by several studies to have a surface temperature of 35˚C-40˚C.[13,14] However, since the temperature of geothermal springs increases with depth, it is possible that the temperature of the source from which water circulates to the surface is higher and in effect may be home to several thermophilic bacteria. In a recent study on generating power by exploiting the geothermal properties of Ikogosi warm spring, the temperature of the spring was recorded to range from 69.45˚C to 71.95˚C.[15] In addition, metagenomics of the spring has revealed the microbial community of the spring to constitute thermophilic bacteria.[14] Despite the evident potential of Ikogosi warm spring, the microbial diversity and hence its biotechnological potential remains largely unexplored. Scientific studies on the spring have majorly been focused on its chemical composition and tourism potential.[13,16] It is essential to intensify the screening for indigenous thermophilic bacteria that may yield thermostable biomolecules with novel properties. This study was therefore aimed at isolating, characterizing, and identifying thermophilic bacteria from Ikogosi warm spring, Ekiti state, Nigeria.

Materials and Methods

Study area

The Ikogosi warm spring is located in Ikogosi, Ekiti state in southwestern Nigeria and is situated at about 2 km west of Ikogosi town on 7° 35' North latitude and 5° 00' East longitude.

Sample collection

Thirty-five (35) samples were collected from six sites along the Ikogosi warm water spring, Ekiti State. Water, sand, and rock samples were collected in sterile capped tubes and transported in an ice chest to the microbiology laboratory at Covenant University, Ota, for further analysis. The samples were preserved at 4˚C prior to further studies. In situ measurements of the pH and temperature were conducted during sampling.

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Isolation of thermophilic bacteria

The enrichment media used for isolation was the Castenholz Tryptone-Yeast Extract (TYE) medium.[19] The media was compounded according to Castenholz, (1969) with components procured from Sigma (USA).[19] One gram of sand samples/1.5mL of water samples/whole-rock were inoculated into 50mL of sterile Castenholz TYE medium and cultivated for 96 hours at 70˚C in a water bath (shaking at 150 rpm). Pure isolates were obtained by streaking the broth culture on Castenholz TYE media supplemented with 3% agar. Culture plates were placed in Ziplocs to avoid drying and incubated at 70˚C for 3-5 days just above the surface of a covered water bath. Pure cultures were then stored in the same media containing 25% glycerol at -80˚C.

Morphological and Biochemical characterization of the isolates

The obtained thermophilic isolates were observed for the following morphological features, namely: cell shape, colonial morphology, colony color, Gram staining, and spore staining. Various biochemical tests such as catalase, oxidase, urease, IMVIC, citrate, and sugar utilization tests were performed for the biochemical characterization of the isolates. The dendrogram of the morphological and biochemical characteristics of the obtained thermophilic isolates was computed using the Principal component analysis (PCA).

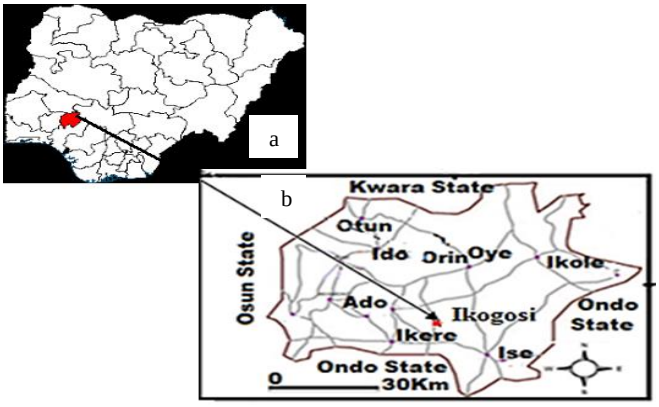

Figure 1: Location of Study.
(a) Map of Nigeria depicting Ekiti State (b) map of Ekiti State depicting Ikogosi (Adapted from Kukoyi, Tijani & Adedara, 2013[17]; Talabi, 2013[18])

Results and Discussion

Thermophilic organisms are heat-loving microorganisms that thrive at temperatures above 45˚C. Their thermostability is speculated to be a result of cellular and subcellular adaptations.[20] In this study, a total of 35 warm spring samples were investigated for the presence of thermophilic bacteria. The pH and surface temperature at the points of sample collection were observed to range from 4.91–5.99 and 33.4˚C-35.5˚C, respectively (Table 1). A total of 27 isolates were obtained on cultivation at 70˚C for 96 hours. The ability of the isolates to grow at such temperatures indicates that they are thermophilic. Similar studies have yielded thermophilic strains on cultivation at the same temperature.[8,21] In this study, all the sample types (water, sand, and rock mattings) yielded thermophilic isolates, and all 27 isolates yielded creamy non-motile colonies. Gupta (2011) has also reported the isolation of thermophilic bacteria from water, sand, and rock mattings obtained from geothermal springs in India.[6] Microscopic characterization of the isolates was carried out using Gram's reaction, spore staining, shape, and motility (Plate 1). Results revealed that out of the 27 isolates, 77.8% were spore formers while 22.2% were non-spore formers. Twenty-three isolates were observed to be Gram-positive, while four isolates were found to be Gram-negative (Table 2). Bergey's manual of systematic bacteriology was used for the identification and classification of the isolates based on their morphological and biochemical characteristics (Table 2). Isolates were observed to be members of the genera *Thermus* and *Geobacillus*.[22] Similar to the results from this study, thermophilic isolates of the genera *Thermus* and *Geobacillus* have been recovered from other hot springs.[21,23] Indeed, Wolella and Tilahun (2020) suggested that *Geobacillus* spp. and *Thermus* spp. are probably the most frequently isolated thermophilic bacteria from hot springs.[8] In the current study, *Geobacillus* spp. were more frequently isolated (85%) than *Thermus* spp. (15%) and this may be due to its ability to form endospores. The dendrogram of the morphological and biochemical characteristics of the 27 thermophilic bacterial isolates from Ikogosi warm spring showed two clusters (A and B). The first cluster (A) included 4 bacterial isolates corresponding to *Thermus* spp. while the second cluster (B) contained 23 bacterial isolates corresponding to *Geobacillus* spp. (Figure 1).

Table 1: Temperature and pH of sample collection site

Point	Temperature (°C)	pH
Point 1	35.5	5.99
Point 2	35	5.96
Point 3	35	5.63
Point 4	33.9	4.91
Point 5	34.5	5.13
Point 6	33.4	4.96

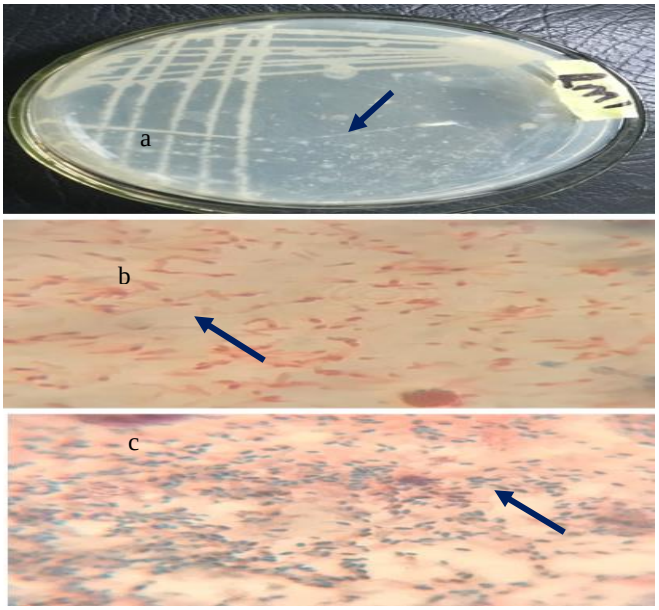

Plate 1: Macroscopic and Microscopic characterization of thermophilic bacteria obtained from Ikogosi warm spring.
(a) Bacterial culture on Castenholz TYE media **(b)** Gram Stain at 100x magnification **(c)** Spore stain

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Table 2: Microscopic and biochemical characteristics of the 27 thermophilic isolates

S/no	Isolate	Microscopic characteristics						IMVIC						Fermentation					
		Color	Texture	Gram's reaction	Shape	Spore formation	Motility	Catalase	Oxidase	Urease	Indole	MR	VP	Citrate	Glucose	Sucrose	Lactose	H_2S	Presumptive Identification
1	W1a	C	S	+	Rods	+	-	+	+	+	-	-	-	-	-	+	-	-	*Geobacillus sp.*
2	W1c	C	S	+	Rods	+	-	+	+	-	-	-	-	-	+	+	-	-	*Geobacillus sp.*
3	S1	C	S	+	Rods	+	-	-	-	+	-	-	-	-	-	-	-	-	*Geobacillus sp.*
4	RM1	C	S	+	Rods	+	-	+	+	-	-	-	-	-	-	+	-	-	*Geobacillus sp.*
5	W2b	C	S	+	Rods	-	-	+	+	+	-	-	-	-	-	+	-	-	*Geobacillus sp.*
6	S2	C	S	+	Rods	+	-	+	+	-	-	-	-	-	+	+	+	-	*Geobacillus sp.*
7	RM2	C	S	+	Rods	-	-	+	-	+	-	-	-	-	+	+	-	-	*Geobacillus sp.*
8	W3b	C	S	-	Rods	-	-	+	+	+	-	-	-	-	+	+	-	-	*Thermus sp.*
9	W3c	C	I	-	Rods	+	-	+	+	+	-	-	-	-	+	+	-	-	*Thermus sp.*
10	S3	C	S	+	Rods	+	-	+	+	+	-	-	-	-	+	+	-	-	*Geobacillus sp.*
11	RM3	C	I	-	Rods	-	-	+	+	+	-	-	-	-	-	+	-	-	*Thermus sp.*
12	W4a	C	S	+	Rods	+	-	+	+	+	-	-	-	-	-	+	-	-	*Geobacillus sp.*
13	W4b	C	S	+	Rods	+	-	+	+	+	-	-	-	-	+	+	+	-	*Geobacillus sp.*
14	S4a	C	I	+	Rods	+	-	+	+	-	-	-	-	-	-	-	-	-	*Geobacillus sp.*
15	S4b	C	I	+	Rods	+	-	+	+	+	-	-	-	-	-	-	-	-	*Geobacillus sp.*
16	W5a	C	S	+	Rods	-	-	+	+	-	-	-	-	-	-	-	-	-	*Geobacillus sp.*
17	S5a	C	S	+	Rods	+	-	+	+	+	-	-	-	-	-	+	-	-	*Geobacillus sp.*
18	S5b	C	S	-	Rods	-	-	+	+	+	-	-	-	-	-	+	+	-	*Thermus sp.*
19	RM5	C	S	+	Rods	+	-	+	+	+	-	-	-	-	-	+	-	-	*Geobacillus sp.*
20	W6a	C	I	+	Rods	+	-	+	+	+	-	-	-	-	-	+	-	-	*Geobacillus sp.*
21	W6b	C	S	+	Rods	+	-	+	+	+	-	-	-	-	-	+	-	-	*Geobacillus sp.*
22	W6c	C	I	+	Rods	+	-	+	+	+	-	-	-	-	+	+	-	-	*Geobacillus sp.*
23	RM6	C	S	+	Rods	+	-	+	+	+	-	-	-	-	-	+	+	-	*Geobacillus sp.*
24	W7a	C	S	+	Rods	+	-	+	+	+	-	-	-	-	+	+	+	-	*Geobacillus sp.*
25	W7b	C	S	+	Rods	+	-	+	+	+	-	-	-	-	+	+	-	-	*Geobacillus sp.*
26	S7	C	S	+	Rods	+	-	+	+	+	-	-	-	-	+	+	-	-	*Geobacillus sp.*
27	S8	C	S	+	Rods	+	-	+	+	+	-	-	-	-	-	+	-	-	*Geobacillus sp.*

Key: Isolates **W**: isolates from water samples; **S**: Isolates from sand; **RM**: isolates from rock matting; Color **C**: creamy; Texture **S**: Smooth, **I**: Irregular

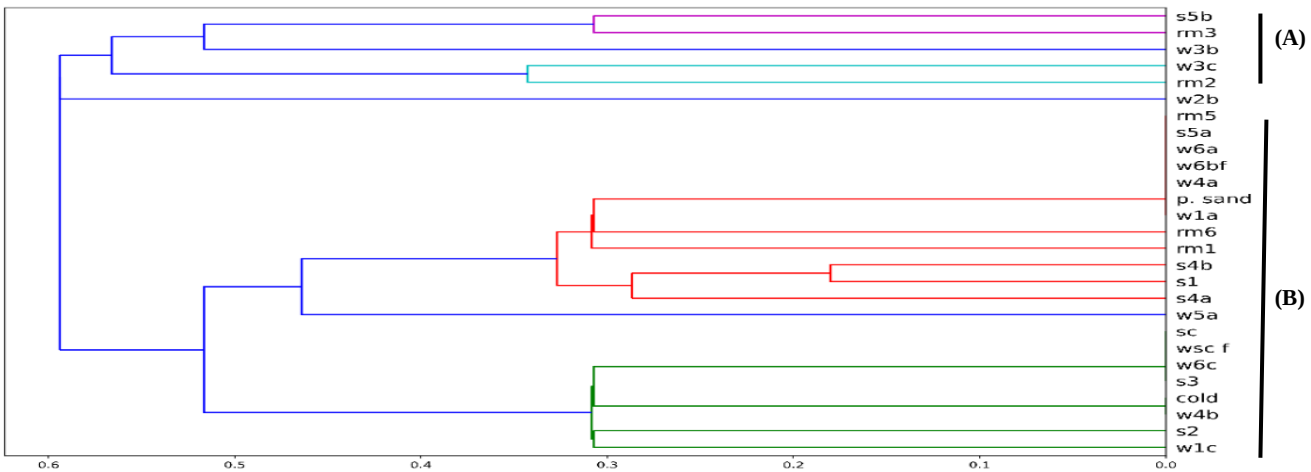

Figure 2: Dendrogram of the morphological and biochemical characteristics of 27 bacterial isolates from Ikogosi warm spring constructed using Principal Component Analysis (PCA). **(A)** Four bacterial isolates corresponding to *Thermus* spp. **(B)** Twenty-three bacterial isolates corresponding to *Geobacillus* spp.

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Conclusion

This study revealed the presence of thermophilic bacteria in Ikogosi warm spring in Ekiti state, Nigeria. Twenty-seven (27) thermophilic isolates of the genera *Thermus* and *Geobacillus* were isolated and characterized. The isolates showed high phenotypic diversity, which may imply diverse industrial and environmental applications.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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