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Research Article

Isolation and Characterization of Extremophilies from Shalla/Abidjata Hot Springs, Ethiopia

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Abstract

The purpose of this study was to isolate and characterize Extremophilic species from geothermal areas of Ethiopian rift valley where the water temperature was 95 - 100 °C and the pH was around 6.5 - 8. About 1.5 ml water and 1 gram sediments were cultured in both Castenholz TYE media and nutrient media. To evaluate the thermo-tolerant characteristics and effects of pH the isolates grew at 60-95 ºC with optimum temperature of at 70 ºC and pH range of 6.0 - 9.5 with optimum pH of 7.5. Three isolates were screened based on the growth characterization at elevated temperature. Isolates NMS and NL2I were aerobic, gram negative, non motile and whitish and TMI were gram positive, motile and whitish. Based on the phenotypic characterization the thermophilic isolate designated as NMS, NL2I and TMI were found to be closely-related to the genus *Thermus* spp and *Bacillus* spp respectively. This study is useful in providing characteristic of indigenous thermopiles that are potential for growth of biotechnology industry.

Keywords: Hot Springs; Extremophiles; *Bacillus* Spp And *Thermus* Spp

Introduction

Extremophilies are microorganisms that can survive and grow under extreme environments, such as high temperature, extreme acidic or basic and high salinity [1].Thermophilicity can be viewed from more than one angle, as there is more than one way in which organisms adapt to thermophilic environments. Extremophilies can develop novel biochemical or physiological properties, such as specific enzyme system, to adapt various extreme environments. The unique cellular structure or stable macromolecules (DNA or proteins) play important roles for extremophiles [2]. Thermophiles are the major part of extremophiles that show extreme tolerance to high temperature. The cellular proteins of thermophiles show higher activity and stability under high temperature. In order to adapt and survive in the extreme environments, thermophiles develop novel properties such as special cell structure, DNA repair system and thermostable enzymes [2].

Extremophiles are very few and definitely limited to the prokaryotic world and can grow optimally at temperatures above 70°C and only a few phylogenetic groups of the Bacteria domain can be considered extremophiles [1,3]. Hot spring is one of the favorable natural geothermal areas which are habitat for most of the thermophiles. Different thermophilic bacteria has been isolated from hot springs and thermal areas like in Yellowstone National Park [4], Icelandic hot spring [5,6], Jordan hot spring [7], Egyptian hot spring [8], Thai hot spring [9], hot water heaters [10], thermally polluted river [11], soil [12], manure pile [13], ocean basin cores [14] and hot composts [15].These thermophilic organisms were found in both neutral and alkaline hot springs [5].

Thermophilic microorganisms have received considerable attention among the scientific community due to their tremendous potential to produce thermostable enzymes and other metabolites that have wide applications in biotechnology, pharmaceuticals and industries [16-19]. Different enzymes are used in molecular and biotechnological research such as hydrolytic enzymes (starch hydrolyzing enzymes, xylose converting enzymes, glucohydrolases, proteases, phosphohydrolases), catalase, DNA processing enzymes (DNA polymerase, DNA ligase, DNA helicases, nucleases), RNA processing enzymes (reverse transcriptases, RNA helicases, ribonucleases) are isolated from thermophilic extremophiles [20].

In different country of the world several indigenous thermophilic species were discovered from hot spring water, geothermal areas and preserved in the indigenous germplasm. In Ethiopia extremophiles are of interest from biotechnological view. Ethiopia is rich in hot spring water. However, such hot water spring microbial diversity has not yet been fully explored due to difficulties in isolation, maintenance of pure culture and thus, their diversity and biotechnological potential remains too unexplored. The aim of this study is, therefore, isolation, identification and characterization of

new thermophilic species from Ethiopian hot water springs. This will be required so as to have indigenous thermophiles that have high biotechnological potentials.

Materials and Methods

Study sites and sampling

The water and sediment samples were collected in triplicates at different points from geothermal areas located about 215 kilometers away from the capital city Addis Abeba, Ethiopia. Shalla/Abidjata Lake is one of Rift Valley lakes in South-Central Ethiopia. Insitu measurement of temperature and pH of Shalla/Abidjata hot springs has been conducted. Shalla/Abidjata comprises of thermal springs with temperatures ranging from 50 to 94°C. The pH value of these springs is from slightly acidic to alkaline (6 - 9.5). The samples were carried using sterile and labeled polythene bottles and stored at about 4°C prior to analysis. This study was conducted in Biotechnology laboratory, Wolkite University in 2018.

Incubation condition and isolation

Aseptic techniques were employed throughout the experiment. One gram of sediments and 1.5 ml of water sample was inoculated in 25 ml of modified Castenholz-TYE media containing 0.1-0.3% tryptone and 0.1 - 0.3% yeast extract or Nutrient Agar (NA) medium. Castenholz-TYE nutrient media had the following composition (in milligrams per liter of deionized water): nitrilotriacetic acid, 100; $CaSO_4$. $2H_20$, 60; $MgSO_4$. $7H_20$, 100; NaCl, 8; KNO_3, 103; $NaNO_3$, 689; Na_2HPO_4, 111; $FeCI_3$, 2.8; $MnSO_4$ H_20, 22; $ZnSO_4$.$7H_20$, 5; H_3BO_3, 5; $CUSO_4$, 0.16; Na_2MoO_4.$2H_20$, 0.25; $CoC1_2$.$6H_20$, 0.46; pH adjusted to 7.4 ± 0.2. After inoculation, the cultures were incubated in 70 °C water bath (shaking at 120 r.p.m.). The temperatures of the water baths was checked frequently with thermometers. Turbid cultures were serially diluted every two days by adding 1.5 ml of the old sample into a freshly autoclaved 25 ml of nutrient media. The original cultures were kept at 4°C refrigerator as stock of back-up cultures in the event that the cultures in the water bath failed to grow. Pure cultures were isolated by streaking samples of the enrichments on plates containing the same medium solidified with 3.0% agar and nutrient agar. The cultures were incubated by fully wrapping in Saran Wrap to prevent drying and incubated just above the surface of the water in a covered water bath. Cultures were then subculturing and preserved at 70°C in the same medium containing 15% glycerol.

Effects of temperature and pH

The effect of temperature for growth was determined by using 0.5ml from overnight cultures as inoculates in 10ml medium. The test tubes were incubated and shaken in covered water baths at 65, 70, 75, 85 and 95°C for 48 hours and growth was determined by their turbidity using spectrophotometer at 600nm. All the tests were made in triplicate. The thermo-tolerance isolates that could tolerate the higher temperature was selected for phenotypic and biochemical characterization. The effects of pH on the growth of thermopiles were also evaluated by growing the inoculants at the pH of 6 to 9.5 with optimum temperature for 48 hours.

Identification and characterization of the isolates

Determinations of genus of thermophilic isolates in this study were in accordance with Bergey's Manual of Determinative Bacteriology. The selected strain was observed morphologically and growth characteristics were studied. Apart from gram staining technique various biochemical tests like endospore formation, motility, and aerobic tests were performed [6,21,22]. Morphological, microscopic observation and biochemical test indicated the bacterium to be *Bacillus* sp.

Antibiotic and inhibitor sensitivity

The Inocula of Thermopiles were grown overnight in capped tubes of liquid media. From this, 0.1ml culture was uniformly spread onto Castenholz-TYE agar plates. Filter paper discs containing measured amounts of antibiotics were then placed onto the surface of the plates. Growth on each plate was checked after further incubation at 70°C for 48 hours. Penicillin, Novobiocin, Actinomycin D and vancomycin sensitivities were determined by placing filter paper discs containing measured amounts of the antibiotic onto agar plates. The diameters of yielded clear zones were estimated visually.

Results

Isolation of thermopiles

Ten thermophilic isolates were obtained from hot spring of Shalla/Abidjata, Ethiopia. Of this thermophilic isolates, three isolates were screened based on their growth stability upon incubation at 70°C for 48 hours. Three of the thermophilic strains were designated as isolate NMS, NL2I and TMI. On the basis of morphological, and biochemical characteristics, isolate NMS, and NL2I are described as *Thermus* spp and TMI as *Bacillus* spp (Table 1 and Figure1).

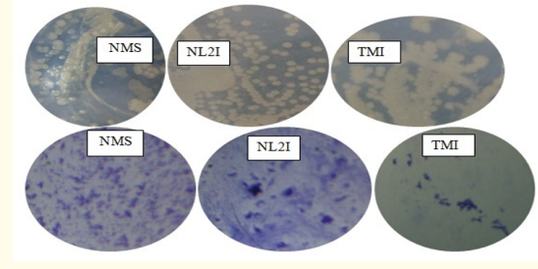

Figure 1: Macroscopic and Microscopic characterization of the thermophilic isolates from Shalla/Abidjata Hot Spring.

Characterization/features	Isolate code		
	NMS	NL2I	TMI
Colony Shape	Small, circular	Small, circular	Small, Round
Colony Texture	shiny	shiny	shiny
Colony Surface	Circular	Circular	Flat
Color of the colony	White-gray	Creamish-white	White
Elevation	Raised	Flat	Flat
Gram reaction	Negative	Negative	Positive
Spore formation	Negative	Negative	Positive
Motility	Non motile	Non motile	Motile
Nitrate reduction	Positive	Positive	Negative
Oxidase	Positive	Positive	Positive
Catalase	Positive	Positive	Positive
Starch hydrolysis	Negative	Negative	Positive

Table 1: Characteristics of the thermophilic isolates.

Effects of temperature and pH on growth profile of thermopiles

The effect of these physiological factors has been investigated. The growth rates of isolated thermophiles were determined at the temperature range of 60 - 95°C and pH of 6 - 9.5. The optimal growth temperature of the isolates was about 70°C (Figure 2). The growth for three of the isolates was poor below 60°C, and above 85 °C and at pH below 6 and above 7.5. The optimum pH was 7.5 for three of the isolates (Figure 3). Therefore the isolates could be both thermopiles and alkaliphiles.

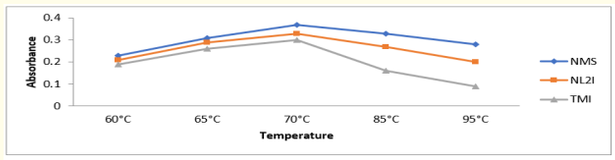

Figure 2: Effects of temperature on the growth of thermophiles.

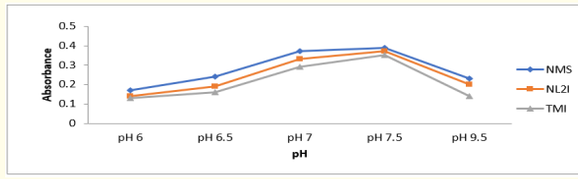

Figure 3: Effects of pH on the growth of thermophiles.

Determination of antibiotic sensitivity of the thermophilic isolates

Among the tested antibiotics strain NMS and NL2I were sensitive to penicillin, Neomycin, and actinomycin D, and resistance to Novobiocin while strain TMI was resistant to penicillin, Neomycin, and actinomycin D and susceptible to Novobiocin.

Discussion

In this study liquid and sediment samples were collected from hot spring of Shalla/Abidjata for thermopiles isolation. Castenholz-TYE medium is a preference media for isolation of thermophiles from hot springs [4,23]. The isolate NMS and NL2I were white and had similar morphological and biochemical characteristics with yellow, orange and non pigmented *Thermus* species [4,24-29]. In line with this study white *Thermus* was isolated from hot springs [30,31]. Isolate TMI had most likely similar morphological and microscopic characteristic of the *Bacillus* spp [32,33,34]. Based on these findings and with the help of Bergeys Manual, thermophilic isolate NMS, NL2I and TMI were identified as *Thermus* spp. and *Bacillus* spp respectively.

The environmental conditions such as temperature and the nutritional status of the hot springs might be the possibility of isolation and characterization of different thermophilic species from different hot springs. Geothermal environments such as hot springs with water temperatures higher than about 55 °C and pH ranging from slightly acidic to alkaline were an ecological niche for thermophiles isolations throughout the world [5,35-37]. Members of the genus *Bacillus* [7,38] and *Thermus* [4-6,9,39] are probably the most frequently isolated thermophiles in the hot environments because of their resistance to harsh environmental conditions. These thermophilic microorganisms are active at elevated temperature because of their resistance due to the enzymes [2,16,18,19].

The growth rate of isolate NMS is slightly faster than isolate NL2I and TMI at the temperature range of 60 - 95 °C and pH of 6-9.5 (Figure 2 and 3). Isolate NMS, NL2I and TMI had nearly the same optimum temperature and pH at 70 °C and 7.5 respectively. The thermophilic *Bacillus* spp [40] and *Thermus* spp [41] grew optimally at temperature of 70~75°C. The thermo tolerance of thermo-

philic species could be influenced by their fatty acid composition [30]. Observation of pH requirement of the thermophilic isolate NMS, NL_2I and TMI indicated that the optimum pH was in range of 7-7.5. In line with this study thermophilic bacteria could not grow in extreme conditions at pH of less than 4.5 and more than 10.5 but it grew at optimum pH between 7.5 and 8.5 [42,43].

The thermophilic isolate NMS and NL2I are all highly sensitive to penicillin, Neomycin, and actinomycin D, and resistance to Novobiocin, as in the case with *Thermus* species [4,40] while strain TMI was resistant to penicillin, Neomycin, and actinomycin D and susceptible to Novobiocin [4,44].

Conclusion

Based on the result of study, three thermophilic NMS, NL2I and TMI strain were isolated and characterized. These thermophiles belong to bacteria *Thermus* and *Bacillus* species. This is the first report on isolation of thermophilic strains from Shalla/Abidjata hot springs. In this study characterization and identification of the thermophilic isolates were made by using phenotypic and biochemical characters. Genotype and fatty acid analysis should be conducted to complete a profile of the thermophilic microbial community of Shalla/Abidjata hot springs. This promising result, provides an evidence that Shalla/Abidjata hot springs supports the growth of *Bacillus* and *Thermus*, widen the opportunities for biotechnological application.

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