Isolation of Actinomycetes from Geothermal Vents of Menengai Crater in Kenya

Abstract

The current study was carried out to isolate actinomycetes from the geothermal vents of Menengai crater. Soil samples were collected from vents A and D in sterile polythene papers and transported to Egerton University, Department of Biological sciences laboratories. The samples were air dried on the benches for one week. To kill vegetative bacteria, the soil samples were heat in a hot air oven for 1h before serial dilution to 10-6. The samples were separately plated on Starch casein agar, Luria Bertani agar and starch nitrate agar in which nystatin and nalidixic acid had been added to reduce the growth of fungi and other types of bacteria. Incubation was carried out at 30oC for up to a period of one Month. The isolated actinomycetes were characterized by cultural, morphological and biochemical means. There was no significant difference in the number of actinomycetes isolated between vents A and vents D (P=0.439). However, the number of actinomycetes isolated using the three isolation media varied significantly (F=37, P=0.03). Totally, 16 actinomycetes were isolated from the vents. It is recommended that the isolates be tested for antagonism against pathogenic microorganism.

Keywords: Actinomycetes; Crater; Geothermal; Isolation; Menengai; Vents

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Introduction

Actinomycetes are comprised of diverse group of organisms that are Gram positive with guanine plus cytosine content in their DNA being greater than 55M % [1]. The term actinomycetes is coined from two greek words, *atkis* meaning ray and *mykes*meaning fungus [2]. This is because the organisms have both fungi and bacteria like features. Like fungi, they form mycelia network branching filaments while like bacteria they have thin cell wall made of muramic acid [3].

Actinomycetes have been used in biodegradation of wastes due to their ability to produce enzymes [4]. These enzymes are capable of decomposing organic compounds. They have also gained popularity in degradation of hydrophobic compounds since they are able to produce biosurfactants such as mycolic acid [5]. Currently, anthropogenic activities such as exploration of petroleum, waste disposal and corrosion of metals have led to accumulation of heavy metals in the environment [6]. This has raised allot of environmental issues which are accrued to the rise of cancer cases today [7]. Actinomycetes are proving to be a great solution to this problem through their ability to biodegrade most if not all of these pollutants [8]. Their promising production of novel drugs that have antitumor properties could help in treating cancer. In addition, the drugs could also be a solution to drug resistant an issue that in causing sleepless nights to most scientists today [9].

Actinomycetes are diverse in nature. They form a distinct evolutionary life when compared to other organisms [10]. They are found in most environments occurring in alkaline soils, desert soils, soils from salt pans, under snow caps, lake water, lake bottoms and in composts peats. In addition, actinomycetes are found in foodstuffs such as fruits, vegetables, milk and milk products and in cacao [11]. Their habitats are grouped into different categories such as marine, desert, mangrove, volcanic areas, those that live in other living organisms and those found in compost pits [12]. The ability to thrive in these areas has led to categorization of actinomycetes based on their habitat. Those that live in temperatures of -2°C -15°C are psychrophiles while the ones living in areas with moderate temperature are mesophyles. Moreover, thermophiles actinomycetes survive in 60° -115 °C [13]. Interms of salinity, actinomycetes doing well at 2-5M Nacl are referred to as halophiles. Those surviving at pH of <4 are acidophiles as opposed to the ones found at pH of >9 which are called alkalophillic [14].

According to [15] environmental conditions that are perceived as beyond normal are termed as extreme environments. Factors such as pH, temperature, pressure, salt concentration, radiation, harmful heavy metals and toxic compounds are used in defining extreme areas [16]. Organisms living in extreme environments are called extremophiles. This term extremophile come from a latin word extremus meaning extreme and Greek phyla which means love [17]. Thus an extremophile is an organism that lives in conditions that are either geographically or physically hostile. These areas have characteristics that can negatively disadvantage normal life [18].

Thermophilic actinomycetes are found in temperatures above 70°C [19]. The ability to survive in these environments is pegged on their membrane structure which has membrane lipids that have more saturated and straight fatty acids than mesophiles [20]. This provides the right degree of fluidity needed for membrane function.

Thermophilic actinomycetes are found in vast environments. Desert soils provide such an environment [21]. Desserts are devoid of much of microbial life due to low water activity. Tolerantactinomycetes however dominate deserts. Hot springs are another important habitat of thermophilic actinomycetes [22]. Hot springs are produced by emergence of geothermal heated ground water. A case in point is an actinomycetes coded LA5^T which could grow at 50-75^{o}C in hot springs found in China. In addition, thermal industrial wastes areas are another source of thermophilic actinomycetes [23]. These areas have wastes that are discarded with a stream of hot water. Volcanic areas cannot be left out in this categorization since they provide a good habitat for these groups of actinomycetes [21]. Volcanic areas are characterized by flow of larva that after cooling gives very good sceneries [22]. No much work has been carried out on actinomycetes from these sites. The current study was carried out to isolate and partially characterize actinomycetes from geothermal vents of Menengai crater in Kenya.

Materials and Methods

The study area

This study was carried out in Menengai crater. The crater is located in Nakuru County in the Great Rift Valley in Kenya. Due to volcanic activities in this region, a caldera which stands at an elevation of 2,278m above the sea level was formed. Although the crater is said to be dormant, it has many geothermal vents lying on the raised regions of its flour. The caldera extents over an area of 90 km^2 having a diameter of 12 km. For the purpose of this study, the study area was divided into region A, B, C and D. The geothermal vents are located in region A (vents A) and D (vents D), the regions from which the soil samples were collection.

Collection of soil samples

From the two sampling regions, soil samples were collected from the vents which could visibly be seen releasing steam Figures 1-3. The samples were separately collected from each of the two sampling regions. The samples were separately mixed to make a composite sample followed by packing in sterile polythene papers. The samples were transported to the department of Biological Sciences laboratories in Egerton University. This was followed by spreading of the soil samples separately on the laboratory benches before heating in a hot air oven at 121^{o}C to encourage isolation of rare actinomycetes.

Preparation of culture media

Actinomycetes were cultured on starch casein agar (SCA) (starch 10 g, K_2HPO_4 2 g, KNO_3 2 g, casein 0.3 g, $MgSO_4.7H_2O$ 0.05 g, $CaCO_3$ 0.02 g, $FeSO_4.7H_2O$ 0.01 g, agar 15 g, filtered sea water 1000 ml and pH 7.0±0.1);Luria Bertani (M1) medium (starch 10g, Peptone 2.0g, Yeast Extract 4.0g, Agar 18.0g, distilled water 1000ml, pH; 7.0±0.1) and starch nitrate agar (soluble starch 20.0g, K_2HPO_4 1.0g, KNO_3 2.0g, $MgSO_4$ 0.5G, $CaCO_3$ 3.0g, NaCl 100g, $FeSO_4$0.1g, $MnCl_2$ 0.1g, $ZnSO_4$ 0.1g, Distilled water 100 ml, pH 7.0±0.1). The media were dissolved in distilled water as guided by the manufacturers prior to autoclaving at 121^{o}C for 15 min. The media were supplemented with 25 µg ml^{-1} nystatin do suppress growth of fungi and 10µg ml^{-1} nalidixic acid to minimize growth of Gram negative and some Gram positive bacteria.

Figure 1: One of the vents in vents A.

Figure 2: One of the vent in vent D.

Figure 3: Soil sample collection from one of the vents in vents D.

Isolation of actinomycetes on culture media

Separately1 g of soil sample was added to 9 ml of distilled water in a test tube. The test tubes were shaken in an orbital shaker rotating at 200 rpm for 10 min to release actinomycetes that were strongly attached to the soil particles. Aseptically, serial dilution was carried out up to 10^{-6}.Following this, 0.1ml of each sample was separately plated in the three isolation media using spread plate technique. The plates were incubated at 30°C for up to one month. The growing colonies were identified as actinomycetes using characteristics such as colonies that were tough, leathery, and partially submerged into the agar. Colonies having these characteristics were sub-cultured using yeast extract malt extract agar medium and incubated at 30°C for up to one month. Sub-culturing was carried out until pure cultures were obtained. The pure cultures were preserved in slants and glycerol after coding using letters PAN followed by a number.

Morphological characterization of actinomycetes

The morphological characteristics of the isolates were studied using slide culture technique. The isolated actinomycetes were streaked on a sterile glass slide having M1 medium. The slides were incubated at 37°C for 7 d. Staining with methylene blue was done followed by observation of the slides using the microscope [21].

Biochemical characterization of actinomycetes

a. **Gram's staining:** Crystal violet, gram's iodine, 95% ethyl alcohol and safranin were used in Gram staining. Briefly, the isolated Actinomycetes were separately placed on glass slides using a wire loop. Aseptically, crystal violet was added on the slides and allowed to stand for 1 min. The excess stain was drained off using tap water. Following this, Gram's iodine was added and the preparation allowed to stand for another 1 min. The excess Gram's iodine was removed using running water. Ethyl alcohol was added drop wise followed by washing with running water. A counter stain safranin was added as a counter stain for 45s before observation of the culture under the microscope [5].

b. **Use of API strips:** Biochemical characterization of the isolates was carried out through inoculating large volumes of the actinomycetes into 0.85% Nacl. McFarland units were used in standardizing the inocula. The inocula were applied into the wells of API strips. The strips were incubated at 30°C for up to 7 d[18].

c. **Carbon source utilization:** The isolated actinomycetes were tested for the ability of utilizing D-Glucose, D-Xylose, L-Arabinose, D-Fructose, D-Galactose, Raffinose, D-Mannitol, sucrose, maltose, lactose and cellulose. The isolates were mixed (1% w/v) with the basal medium followed by incubation at 30°C for 7 d.

Data Analysis

The data obtained was analyzed using Statistical package for social sciences (SSPS) version 17.0software. Means of actinomycetes from geothermal vents of region A and D were compared using t-test while comparison of the number of actinomycetes isolated using the three media were compared using ANOVA.

Result

Number of actinomycetes isolated from soils of vents A and D in Menengai crater

The actinomycetes isolates from vents A varied from 5±0.2-1±0.3 when isolated using SC, M1 (6±0.1-16±0.3) and SN (3±0.2-10±0.1) (Table 1). In vents D the ranges were SC (3±0.3-7±0.3), M1 (8±0.1-14±0.1) and SN (6±0.3-8±0.2).

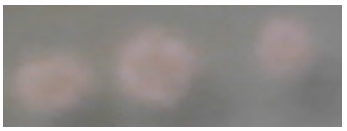
Table 1: Number of actinomycetes isolated from soils of vents A and D in Menengai crater using different media.

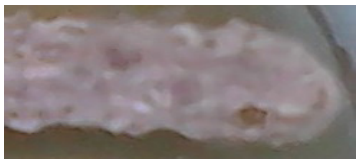
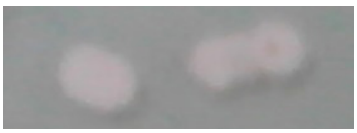
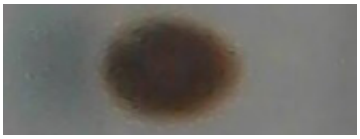
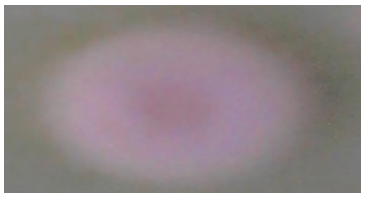
DF	Number of Actinomycetes					
	Vents A			Vents D		
	SC	M1	SN	SC	M1	SN
3-Oct	5±0.2	16±0.3	10±0.1	7±0.3	14±0.1	8±0.2
4-Oct	5±0.1	16±0.3	8±0.2	5±0.2	13±0.1	7±0.1
5-Oct	4±0.3	13±0.1	9±0.3	6±0.1	10±0.2	8±0.1
6-Oct	2±0.1	15±0.2	4±0.1	3±0.2	12±0.3	4±0.3
7-Oct	3±0.2	12±0.1	5±0.2	3±0.3	11±0.3	5±0.2
8-Oct	1±0.3	6±0.1	3±0.2	3±0.3	8±0.1	6±0.3
Mean	3.3±0.2	13±0.1	6.5±0.3	4.5±0.3	11.3±0.1	6.3±0.2

Morphological characteristics of actinomycetes isolated from vents A and D in Menengai crater

A total of 16 actinomycetes were isolated from vents A and D (Table 2). From Vents A, PAN 22 had grey aerial mycelium, cream substrate mycelium, no diffusible or melanin pigments, raised elevation and a rough surface. PAN 34 dark grey aerial mycelium, cream sub-surface mycelium, it produced diffusible pigments but not melanin pigments. It had a raised elevation and a rough surface. PAN 36 produced dark grey aerial mycelium, brown sub-surface mycelium, diffusible pigments but had no melanin pigments production. The isolate had raised elevation and a rough surface. PAN 53 had white aerial mycelium, cream sub-surface mycelium, no diffusible or melanin pigments but had raised elevation with a rough surface. The aerial mycelium of PAN 104 was purple, while the substrate mycelium brown. The isolate had no diffusible or melanin pigments. Its elevation was raised while the surface was rough. PAN 106 had purple aerial and sub-surface mycelia. The isolates had no diffusible or melanin pigments. It had a raised elevation that appeared rough. PAN 136 had orange aerial mycelium and yellow substrate mycelium. The isolate had no diffusible or melanin pigments. It had raised elevation and a smooth surface.

Table 2: Morphological and cultural characteristics of actinomycetes isolated from geothermal vents A and D in Menengai crater.

Vents A	Code	Aerial Mycelium	Substrate Mycelium	Diffusible Pigments	Melanin Pigments	Elevation	Surface	Figure
	PAN 22	Grey	cream	-	-	raised	rough	
	PAN 34	Dark grey	cream	+	-	raised	rough	
	PAN 36	Dark grey	brown	+	-	raised	Rough	
	PAN 53	White	cream	-	-	raised	rough	
	PAN 104	Purple	Brown	-	-	raised	rough	
	PAN 106	Purple	purple	-	-	raised	rough	
	PAN136	Orange	yellow	-	-	raised	Smooth	

	PAN 137	Dark Orange	brown	-	-	flat	smooth	
Vents D	PAN 42	Dark grey	brown	-	-	raised	rough	
	PAN 70	white	cream	-	-	raised	Rough	
	PAN 72	White	white	-	-	raised	rough	
	PAN 132	Black	brown	-	-	raised	rough	
	PAN 153	Transparent	transparent	-	-	raised	Smooth	
	PAN 154	White/ yellow	cream	-	-	raised	rough	
	PAN 155	Brown white	cream	-	-	flat	rough	
	PAN 156	White brown	brown	-	-	flat	rough	

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Lastly, PAN 137 produced peach aerial mycelium and brown sub-surface mycelium. It had neither diffusible nor melanin pigments. The elevation was flat with a smooth surface. On the other hand, from vents D, PAN 42 produced dark grey aerial mycelium with brown sub-surface mycelium. It did not produce either diffusible or melanin pigments. The elevation was raised with a rough surface. PAN 70 had white surface mycelium and cream sub-surface mycelium. The isolate did not produce diffusible of melanin pigments. The elevation was raised and rough. White aerial and sub-surface mycelium was produced by PAN 72. However, the isolate did not produce diffusible or melanin pigments. The surface was raised and rough. PAN 132 had black aerial mycelium and a brown sub-surface mycelium. It did not produce diffusible or melanin pigments. On the other hand PAN 153 produced transparent aerial and sub-surface mycelia. It did not produce diffusible or melanin pigments. It had a raised surface which was smooth. PAN 154 produced white yellow aerial mycelium and cream sub-surface mycelium. There was no diffusible or melanin production. The surface was raised and rough. In addition, PAN 155 had brown white aerial mycelium and cream sub-surface mycelium. The isolate did not produce diffusible or melanin pigments. Its elevation was flat and the surface was rough. PAN 156 had white aerial and brown sub-surface mycelia. It did not produce either diffusible or melanin pigments. The elevation was flat and the surface rough.

Biochemical characteristics of actinomycetes isolated from Menengai crater

All the isolates tested positive for Gram stain, catalase, oxidase, urea hydrolysis and gelatin liquefaction (Table 3). They were all negative for deaminase and Indole production. PAN 104, PAN132 and PAN 155 were positive for beta-glucosidase while PAN 22, PAN 34, PAN 36, PAN 53, PAN 106, PAN 136, PAN 137, PAN 42 and PAN 70, PAN 72, PAN153, PAN154 and PAN 156 were negative. PAN 53, PAN 136, PAN 137, PAN 70, PAN 72 and PAN 156 were positive for lysine decarboxylase. However, PAN 22, PAN 34, PAN 36, PAN 104, PAN 106, PAN 42, PAN 132, PAN 153, PAN 154 and PAN 155 were negative.

The isolates PAN 22, PAN 34, PAN 106, PAN 136, PAN 137, PAN 153, PAN 154 and PAN 156 were positive while PAN 36, PAN 53, PAN 104, PAN 42, PAN70, PAN 72, PAN 132 and 155 were negative for ornithine decarboxylase. In addition, PAN 22, PAN 34, PAN 104, PAN 106, PAN 136, PAN 137, PAN 132, PAN 153, PAN 154, PAN 155 and PAN 156 were positive for citrate utilization. However, PAN 36 and PAN 53, PAN 42, PAN 70 and PAN 70 tested negative for citrate utilization. On production of H_2S, PAN 36, PAN 53, PAN 42, PAN 70, PAN 72, and PAN 154 were positive while PAN 22, PAN 34, PAN 104, PAN 106, PAN 136, PAN 137, PAN 132, PAN 153, PAN 155 and 156 tested negative.

Carbon source utilization of actinomycetes isolated from Menengai Crater

All the isolates utilized D-glucose, D-galactose, sucrose, D-fructose and L-arabinose. In addition, all of them tested negative for D-mannitol, salicin, raffinose and meso-inositol (Table 4). However, PAN 34, PAN 53, PAN 104, PAN 106, PAN 42, PAN 70, PAN 72, PAN 132, PAN 153, PAN 154, PAN 155 and PAN 156 were positive while PAN 22, PAN36, PAN136, PAN137 were negative for lactose utilization. All the isolates utilized maltose apart from PAN 36 and PAN53.

Table 3: Biochemical characteristics of actinomycetes from geothermal vents A and D in Menengai Crater.

Test	Strain															
	Vents A								Vents D							
	PAN	PAN 34	PAN 36	PAN	PAN	PAN 106	PAN	PAN	PAN	PAN 70	PAN 72	PAN	PAN	PAN	PAN	PAN
	22			53	104		136	137	42			132	153	154	155	156
GS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ONPG	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-
CAT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GLU	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LDC	-	-	-	+	-	-	+	+	-	+	+	-	-	-	-	+
ODC	+	+	-	-	-	+	+	+	-	-	-	-	+	+	-	+
CIT	+	+	-	-	+	+	+	+	-	-	-	+	+	+	+	+
H2 S	-	-	+	+	-	-	-	-	+	+	+	-	-	+	-	-
URE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TDA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 4: Carbon source utilization of actinomycetes isolated from vents A and D in Menengai Crater.

Test	Strain															
	Vents A								Vents D							
	PAN	PAN 34	PAN 36	PAN	PAN	PAN 106	PAN	PAN	PAN	PAN	PAN 72	PAN	PAN	PAN	PAN	PAN
	22			53	104		136	137	42	70		132	153	154	155	156
DG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D Gal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cell	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Su	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
DM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lac	-	+	-	+	+	+	-	-	+	+	+	+	+	+	+	+
Mal	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Sal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DF	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Raf	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Discussion

Isolation of actinomycetes from hostile environment such as geothermal sites is important today so as to increase the possibility of getting unique isolates. The number of actinomycetes isolated in this study (Table 1) is higher than that of a previous study [4]. The possible reason could be that the soils of Menengai crater are richer in terms on nutrients. [23] asserts that the soil nutrient level greatly influences the survival of actinomycetes. The morphological characteristics of actinomycetes isolated in the current study are typical of actinomycetes (Table 2). A similar study carried out in Xinjiang yielded similar results [12]. This may have been caused by the two study sites having the same environmental conditions in addition to the use of standard methods in isolating the actinomycetes.

Biochemical characteristics of actinomycetes pray an important role in their classification [7]. The isolates obtained in this study yielded results that are a characteristic of actinomycetes (Table 3). This partly agreed with a study carried out in Egypt [17]. These could have come about due to the isolates been the same. According to [16] same strains of actinomycetes give the same biochemical characteristics due to the isolates following the same biochemical pathways. Carbon source utilization differed with a previous study [20]. The difference could have originated from differences in the study area in terms of the carbon sources present. According to [11] when actinomycetes grow in a certain environment, they get adapted to utilizing the carbon sources present. [14] also asserts that different strains of actinomycetes have different carbon source requirements.

Conclusion

Actinomycetes were isolated from geothermal vents of Menengai crater. The isolates different morphological and biochemical characteristics in addition to varying carbon source requirements. There is need for further analysis to be carried out to establish whether the isolates have antimicrobial properties.

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