Greener Journal of Microbiology and Antimicrobials

ISSN: 2354-2284

Submitted:18/04/2017
Accepted:11/05/2017
Published: 16/05/2017

DOI: http://doi.org/10.15580/GJMA.2017.2.041817051

Isolation of Actinomycetes from the Soils of Menengai Crater in Kenya

By

Paul Njenga Waithaka
Francis B. Mwaura
John M. Wagacha
Eliud M. Gathuru

Research Article (DOI: http://doi.org/10.15580/GJMA.2017.2.041817051)

Isolation of Actinomycetes from the Soils of Menengai Crater in Kenya

Paul Njenga Waithaka[1*], Francis B. Mwaura[1], John M. Wagacha[1] and Eliud M. Gathuru[2]

[1]University of Nairobi, School of Biological Sciences, P. O. Box, 30197-00100 Nairobi, Kenya.
[2]Egerton University, Department of Biological Sciences, P. O. Box, 536 Njoro, Kenya.

*Corresponding Author's Email: waithakanj @gmail. com; Cell: +254 721104637

ABSTRACT

This study was carried out to isolate actinomycetes from the soils of Menengai crater. The study area was divided into regions A, B, C and D. Soil samples were collected from 8 sampling points from each region. The samples were separately mixed to form composite samples. Starch casein, Luria Bertani and starch nitrate agar were used in isolating actinomycetes using spread plate technique. Prior to isolation, the soil samples were heat for 1h at 121ºC followed by serial dilution upto 10^{-6}. The isolates were characterized using cultural, morphological and biochemical means. Region B and C produced many actinomycetes than A and D. Luria Bertani agar was better in recovering actinomycetes than starch casein and starch nitrate agar. There was significant difference in the number of actinomycetes isolated using the three media (F=3.315 P=0.04218). The number of actinomycetes isolated from region A, B C, and D also varied significantly (F= 27.50 P=0.000). A total of 152 actinomycetes were isolated. There is need to test the actinomycetes isolates for production of antimicrobials.

Keywords: actinomycetes, isolation, Kenya, Luria Bertani, Menengai, Starch casein.

INTRODUCTION

For long, actinomycetes have been thought to be fungi. However, current studies have shown that indeed they are bacteria (Prasannan *et al.,* 2016). As a result, taxonomists have placed them between true bacteria and true fungi. This has been inspired by an in depth study of their cell wall coupled with other characteristics (Modi *et al*., 2016).

Actinomycetes inhabit a wide range of ecological zones (Chavan *et al.,* 2013). Despite this, they are known to be ambiquitours free living, filamentous microorgnaisms with their main home being the soil. In addition, they are gram positive, saprophytic bacteria (Atsuko and Takahashi, 2017). The GC content in their DNA is more than 55% a feature that makes them unique compared with other microorganisms (Siddique *et al.,* 2014).

In the soil, they are involved in a symbiotic relationship with plants besides praying a key role in nutrient cycling (Zhang, *et al.,* 2014). Actinomycetes are also involved in degradation of polythenes in damping sites. The organisms are also abundant in water bodies where they help in biodegradation of industrial effluents dumped from industries (Zakalyukina *et al.,* 2017). By doing this, they help in preservation of aquatic flora and fauna (Prashith *et al*., 2015).

According to Ashokvardhan and Satyaprasad (2016), when found in any other environment away from soil, actinomycetes must have been taken there by various agents such as floods. In their natural habitat actinomycetes are affected by soil pH, temperature, soil texture, ploughing, organic matter content, amount of oxygen in the soil and the cation exchange capacity of the soil (Varalakshmi *et al*., 2014).

Actinomycetes are specifically unique in their wide range of metabolites production (Manojkumar and Subbaiya, 2016). They produce vitamins, herbicides, antihelminthes, immunosuppressants, enzymes, nutritional supplements and most significantly, antibiotics. Production of these products varies depending on their habitat (Janaki *et al*., 2016). Currently, over 5000 antibiotics have been screened from actinomycetes for use in human, animal and plant disease therapy. Over 60% of the known antibiotics have been produced from Streptomycetes while 15% come from rare actinomycetes such as Micromonospora, Actinomadura, Streptoverticillum and Thermoactionmycetes that have varying morphological characteristics (Wadetwar and Patil, 2013). The aim of this study was to isolate and partially characterize actinomycetes from soils of Menengai crater.

MATERIALS AND METHODS

The study area

The current study was carried out in Menengai crater which is located to the North of Nakuru town in Kenya. The crater is a product of a volcanic eruption which took place 200,000 years ago. The eruptions lead to formation of a caldera that rises 2,278m above the sea level. The flour of the crater is characterized by steams which come from underground geothermal vents. However, the crater is dormant but speculations maintain that it may turn active after every 50 years. Though these are mare speculations, the shaky grounds around the crater attest to this fact.

The caldera extends over an area of 90 km^2 with a diameter of 12 km. In Africa, Menengai comes second after Ngorongoro of Tanzania. In terms of location, the crater is located in Rongai and Nakuru North Sub-counties at 35º 28', 35º 36'E, and 0º 13', 1º 10'S (Omenda *et al*., 2000).

Soil sample collection

The study area was divided into four regions viz A, B, D and D and 8 sampling points were randomly identified from each region. From each of the four regions, 8 two hundred grams sub-samples were collected from the top 5 cm. The samples from each of the regions were mixed to form a composite sample. The samples were separately packed in sterile polythene bags and transported to Egerton University, Department of Biological Sciences laboratories. In the laboratory the samples were separately air dried on the benches for one week to help reduce the population of gram negative bacteria (Khasabuli and Kibera, 2014). The samples were separately sieved through 250 µm pore size sieve (United Kingdom). Heat treatment was carried out by separately placing the samples in a hot air oven at 121ºC for 1h to prevent growth of other bacterial flora.

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 (Soluble 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_4HPO: 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 per the manufacturer's instructions before autoclaving at 121ºC for 15 min. Following this, the media were supplemented with 25 µg ml^{-1} nystatin and 10µg ml^{-1} nalidixic acid to minimize contamination with fungi and bacteria species respectively.

Isolation of actinomycetes on culture media

From each of the composite samples, 1 g of soil sample was separately added to a test tube containing 9 ml distilled water and shaken vigorously at room temperature (25 ± 2ºC), using an orbital shaker at 200 rpm for 10 min. The test tubes were considered as stock culture for the soil sample. Aseptically, 1 ml aliquots from the stock solutions were separately transferred to a test tube containing 9 ml of sterile distilled water and mixed well. From these test tubes, 1 ml of aliquots were transferred and mixed with another 9 ml of distilled water to make 10^{-2} dilution factor. Similarly, dilutions up to 10^{-6} were made using serial dilution technique for all soil samples (Saravana *et al*., 2014).

After serial dilution, 0.1ml of each sample was separately plated in the three isolation media using spread plate technique. The plates were incubated at 28ºC, and observed from 5th day onwards for 25 days. After incubation, actinomycete isolates were distinguished from other microbial colonies by characteristics such as tough, leathery colonies which are partially submerged into the agar (Wang *et al*., 2016). Colonies with suspected actinomycetes morphology were be sub-cultured on yeast extract malt extract agar medium and incubated at 28ºC for 5 to 25 days. Pure cultures were inoculated in 10 ml of yeast extract malt extract broth (YMB) and incubated at room temperature (25 ± 2ºC) for 24 to 48 h in a rotary shaker (200 rpm). The pure cultures were maintained in slant culture on yeast extract malt extract agar (ISP2) as well as in glycerol broth at 4ºC for further studies (Rajan *et al*., 2014).

Morphological characterization of actinomycetes

Identification of the actinomycetes was carried out using cover slip method. The morphological characteristics of the isolates were observed under the microscope . M1 agar was placed on sterile glass slides . The isolated

actinomycetes were streaked on the slide prior to incubation at 37°C for 7 days. Two drops of methylene blue were added followed by observation of the slides using the microscope (Ajunwa *et al*., 2016).

Biochemical characterization of actinomycetes

Gram's staining

Crystal violet, gram's iodine, 95% ethyl alcohol and safranin were used in Gram staining. Briefly, Actinomycetes were separately placed on glass slides using a wire loop. Crystal violet was added and allowed to stand for 1 min. Excess stain was removed using running water. 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 dropwise followed by washing with water. Safranin was used as a counter stain for 45s prior to microscopic examination of the culture (Cassir *et al.,* 2014).

Use of API strips

Large volumes of the actinomycetes colony were separately inoculated into 0.85% Nacl solution. McFarland barium sulfate was used in standardizing the inocula which were then inoculated into the wells of API strips. The strips were incubated at 30ºC for up to 7 d (Humera *et al.,* 2016).

Carbon source utilization

Tests were carried out to determine the ability of the isolates to utilize carbon as recommended by International *Streptomyces* Project (ISP) (Duddu *et al.,* 2016). The carbon sources (1% w/v) such as D-Glucose, D-Xylose, L-Arabinose, D-Fructose, D-Galactose, Raffinose, D-Mannitol, Sucrose, Maltose, Lactose, Cellulose were mixed (1% w/v) with the basal medium. The actinomycetes isolates were streaked on the medium and incubated at 30ºC for 7 d.

Coding of the isolates

Isolates bearing the same morphological, cultural and biochemical characteristics were grouped together and coded PAN followed by a number.

Data analysis

All data analyses procedures were carried out using statistical package for social sciences (SSPS) version 17.0 software. Comparisons of the means between regions A, B, C and D and between the isolation media were carried out using one way ANOVA.

RESULTS

In region A, the actinomycetes isolates varied from $2\pm3x10^8$ to $9\pm2x10^3$ in SC, M1 ($5\pm2x10^8$-$14\pm3x10^3$) and SN ($4\pm3x10^8$-$14\pm2x10^3$) (Table 1). On the other hand, the variation of actinomycetes isolates in region B were Sc ($15\pm2x10^8$-$25\pm3x10^3$), M1 ($20\pm3x10^8$-$30\pm2x10^3$) and SN ($14\pm2x10^8$-$27\pm3x10^3$). In addition, actinomycetes isolated from region C ranged from ($16\pm2x10^8$-$24\pm2x10^3$) in SC, M1 ($20\pm2x10^8$- $31\pm3x10^3$) and SN ($17\pm3x10^8$-$28\pm2x10^3$). Besides, Actinomycetes ranges in region D were SC ($5\pm2x10^8$-$9\pm2x10^3$), M1 ($7\pm2x10^8$-$15\pm3x10^3$) and SN ($4\pm3x10^8$-$13\pm3x10^3$). There was a significant difference in the number of actinomycetes isolated using the three media ($F=3.315$ $P=0.04218$). Likewise, the number of actinomycetes isolated from region A, B C, and D varied significantly ($F= 27.50$ $P=0.000$).

A total of 152 actinomycetes having varying morphological and cultural characteristics were isolates from soils of Menengai crater (Table 2). PAN 1-20 showed green aerial mycelium and yellow substrate mycelium. The isolates did not produce diffusible or melanin pigments in addition to having a flat elevation and a rough surface. However PAN 21-34 had grey aerial mycelium and cream substrate mycelium. The isolates didn't produce any diffusible or melanin pigments but the surface was flat and rough. PAN 35-50 had a dark grey surface mycelium with a cream substrate mycelium. The isolates produced brown diffusible pigments but no melanin pigments. Their surfaces were raised and rough. PAN 51-81 were white with a cream substrate mycelium. They produced neither diffusible nor melanin pigments. Their surface was raised and rough.

Conversely, PAN81-100 were violet in both aerial and substrate mycelium. They had violet diffusible pigments with no melanin pigments while their surface was raised. Additionally, PAN 101-105 had a purple surface with a black subsurface mycelium. The isolates had brown diffusible pigments but no melanin pigments. Their surface was flat and rough. PAN 106-116 were light brown with brown substrate mycelia. Their diffusible pigments were brown with no melanin pigments. The surface was flat and smooth. PAN117-120 had dark brown aerial and brown subsurface mycelium with brown diffusible pigments but no melanin pigments. The isolates had a flat surface that was rough. In PAN121-126, the aerial mycelia were blue while the substrate mycelia were black. The isolates did not produce either diffusible of melanin pigments. They had a surface that was raised and rough. The isolates PAN127-130 produced dark blue aerial mycelium and black substrate mycelium. Additionally, they produced brown diffusible and melanin pigments. Their surface was raised and rough. In PAN131-133, their aerial and substrate mycelia were black. They produced no diffusible and melanin pigments. Their surface was raised and rough. PAN134-138 had an orange surface mycelium with yellow subsurface mycelium. Their surface was raised and rough. On the other hand, PAN 139-152 had peach surface mycelium with no diffusible and melanin pigments production. Their surface was flat and rough.

Biochemical characteristics of actinomycetes isolated from Menengai crater

All the isolates tested positive for Gram stain, catalase, oxidase, urea hydrolysis and gelatin liquefication (Table 3). They were all negative for deaminase and Indole production. PAN 101-105, PAN121-126 and PAN 139-152 were positive for beta-galactosidase while PAN 1-20, PAN 21-34, PAN 35-50, PAN 51-81, PAN 106-116, PAN 117-120, PAN 127-130, PAN 131-133 and PAN 134-138 were negative. PAN 1-20, PAN 51-81, PAN 117-120 and PAN 134-138 were positive for lysine decarboxylase.

PAN 21-34, PAN 35-50, PAN 81-100, PAN 101-105, PAN 106-116, PAN 121-126, PAN 127-130, PAN 131-133 and PAN 139-152 were negative for lysine decarboxylase. The isolates PAN 21-34, PAN 106-116, PAN 117-120, PAN 131-133, PAN 134-138, PAN 134-138, PAN 139-152 were positive while PAN 1-20, PAN 35-50, PAN 51-81, PAN 81-100, PAN101-105, PAN 121-126, PAN 127-130 were negative for ornithine decarboxylase.

In addition, PAN 1-20, PAN 21-24, PAN81-100, PAN 101-105, PAN 105-116, PAN 117-120, PAN 121-126, PAN 127-130, PAN 131-133, PAN 134-138 and PAN 139-152 were positive for citrate utilization. Only PAN 35-50 and PAN 51-81 tested negative for citrate utilization. When tested for H_2S production, PAN 35-50, PAN 51-81, PAN 117-120, PAN 121-126, PAN 131-133 were positive while PAN 1-20, PAN 21-34, PAN 81-100, PAN 101-105, PAN 106-116, PAN 127-130, PAN 134-138, PAN 139-152 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 could not utilize cellulose, D-mannitol, salicin, raffinose and meso-inositol (Table 4). However, all the isolates apart from PAN 35-50 could utilize sucrose. PAN 21-34, PAN 51-80, PAN 81-100, PAN 101-105, PAN 121-126, PAN 127-130, PAN 131-133, PAN 134-138, PAN 139-152 were positive while PAN1-20, PAN35-50, PAN106-116, PAN117-120 were negative for lactose utilization. All the isolates utilized maltose apart from PAN 35-50 and PAN51-80.

DISCUSSION

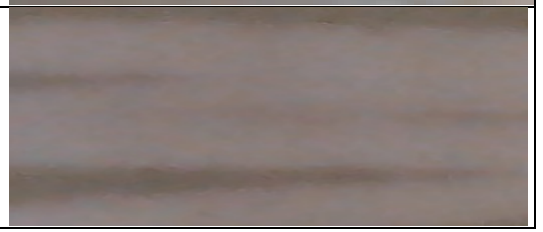
The current study indicates that Menengai crater is a fertile ground for isolation of actinomycetes (Table 1). Region B and C produced the highest number of actinomycetes compared to regions A and D. This can be attributed to the topography of the crater where region B and C are located on the lower side while regions A and D are on the upper side. This agreed with a previous study carried out in semi-arid regions of North Africa (José *et al*., 2017). As a result most of the actinomycetes could have been transported to the lower regions through soil erosion (Surabhi, and Srividya, 2016).

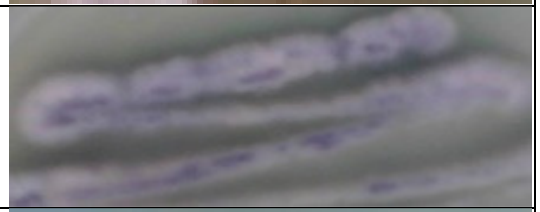
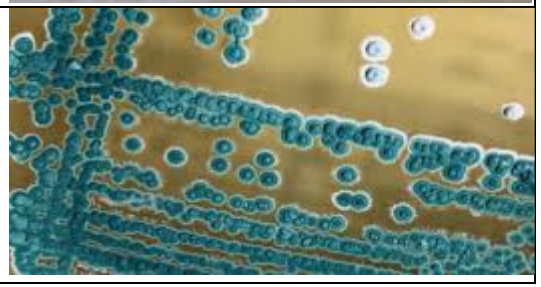
Table 1: Number of actinomycetes isolated from soils of Menengai crater using different media

DF	Number of actinomycetes											
	Region A			Region B			Region C			Region D		
	SC	M1	SN	SC	M1	SN	SC	M1	SN	SC	M1	SN
10^{-3}	9±2	14±3	14±2	25±3	30±2	27±3	24±2	31±3	28±2	9±2	15±3	13±3
10^{-4}	6±2	12±2	10±2	21±3	30±2	24±3	22±2	31±2	25±2	7±3	11±3	10±3
10^{-5}	4±3	10±2	7±2	19±2	28±3	21±3	22±2	30±3	22±3	5±2	10±3	11±2
10^{-6}	3±2	8±3	8±3	18±2	26±2	18±3	17±3	24±2	21±3	6±2	10±2	5±2
10^{-7}	3±3	7±2	5±2	15±3	27±3	15±2	18±3	22±2	19±2	5±2	9±2	3±2
10^{-8}	2±3	5±3	4±3	15±2	20±3	14±2	16±2	20±2	17±3	5±2	7±2	4±3
Mean	**4.5**±3	**9.3**±2	**8.0**±2	**18.8**±2	**27.3**±3	**19.8±3**	**19.8±3**	**27.0**±2	**22.0**±3	**6.2±2**	**10.3**±3	**7.67**±3

DF; dilution factor, SC; starch casein, M1; Luria Bertani, SN; starch nitrate.

Table 2: Morphological and cultural characteristics of actinomycetes isolated from Menengai crater

Code	Aerial mycelium	Substrate mycelium	Diffusible pigments	Melanin pigments	Elevation	Surface	
PAN 1-20	Green	Yellow	-	-	flat	rough	
PAN 21-34	Grey	Cream	-	-	flat	rough	
PAN 35-50	Dark grey	cream	+	-	Raised	rough	

PAN 51-80	White	cream	-	-	raised	rough	
PAN81-100	Violet	violet	+	-	raised	rough	
PAN 101-105	purple	Black	+	-	flat	rough	
PAN 106-116	Light brown	Brown	+	-	flat	smooth	
PAN117-120	Dark brown	Brown	+	-	flat	rough	
PAN121-126	Blue	black	-	-	raised	rough	

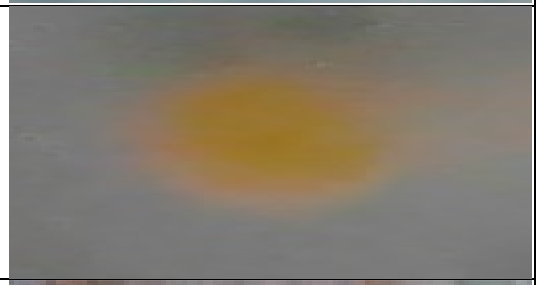
PAN127-130	Dark Blue	black	+	+	raised	rough	
PAN131-133	Black	black	-	-	raised	rough	
PAN134-138	Orange	yellow	-	-	raised	smooth	
PAN 139-152	Peach	cream	-	-	flat	rough	

Table 3: Biochemical characteristics of actinomycetes isolated from Menengai Crater

Test	Strain												
	PAN 1-20	PAN 21-34	PAN 35-50	PAN 51-81	PAN 81-100	PAN 101-105	PAN 106-116	PAN 117-120	PAN 121-126	PAN 127-130	PAN 131-133	PAN 134-138	PAN 139-152
GS	+	+	+	+	+	+	+	+	+	+	+	+	+
ONPG	-	-	-	-	-	+	-	-	+	-	-	-	+
CAT	+	+	+	+	+	+	+	+	+	+	+	+	+
GLU	+	+	+	+	+	+	+	+	+	+	+	+	+
LDC	+	-	-	+	-	-	-	+	-	-	-	+	-
ODC	-	+	-	-	-	-	+	+	-	-	+	+	+
CIT	+	+	-	-	+	+	+	+	+	+	+	+	+
H_2 S	-	-	+	+	-	-	-	+	+	-	+	-	-
URE	+	+	+	+	+	+	+	+	+	+	+	+	+
TDA	-	-	-	-	-	-	-	-	-	-	-	-	-
IND	-	-	-	-	-	-	-	-	-	-	-	-	-
GL	+	+	+	+	+	+	+	+	+	+	+	+	+

GS: Gram stain, ONPG: beta-galactosidase, CAT: catalase test, GLU: oxidase, LDC: lysine decarboxylase, ODC: ornithine decarboxylase, CIT: citrate utilization, H_2 S: Hydrogen sulphide production, URE: urea hydrolysis, TDA: deaminase, IND: Indole production, GL: Gelatin liquefication.

Table 4. Carbon source utilization of actinomycetes isolated from Menengai Crater

Test	Strain												
	PAN 1-20	PAN 21-34	PAN 35-50	PAN 51-80	PAN 81-100	PAN 101-105	PAN 106-116	PAN 117-120	PAN 121-126	PAN 127-130	PAN 131-133	PAN 134-138	PAN 139-152
DG	+	+	+	+	+	+	+	+	+	+	+	+	+
DGal	+	+	+	+	+	+	+	+	+	+	+	+	+
Cellulose	-	-	-	-	-	-	-	-	-	-	-	-	-
Su	+	+	-	+	+	+	+	+	+	+	+	+	+
DM	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	-	+	-	+	+	+	-	-	+	+	+	+	+
Mal	+	+	-	-	+	+	+	+	+	+	+	+	+
Salicin	-	-	-	-	-	-	-	-	-	-	-	-	-
DF	+	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-
Meso	-	-	-	-	-	-	-	-	-	-	-	-	-
LA	+	+	+	+	+	+	+	+	+	+	+	+	+

DG;D-Glucose, DGal; D-Galactose, Su; sucrose, DM; D-Mannitol, Mal; Maltose, DF; D-Fructose, Meso; Meso-Inositol, LA; L-Arabinose.

M1 agar was able to recover higher number of actinomycetes than either starch casein and starch nitrate agar (Table 1). This differs with earlier studies carried out in other parts of the word (Atta, 2015; Qais *et al*., 2017). The hostility of the crater in terms of growth conditions of actinomycetes may be a contributing factor. Slam *et al.* (2014) asserts that the growth conditions that actinomycetes are subjected to may results in change in nutrient requirements.

The isolates obtained had varying cultural and morphological characteristics (Table 2). This agrees with a previous study carried out in Kericho (Rotich *et al*., 2017). However, the isolates differed in some details such as the colony colour, elevation and surface morphology (Kumari *et al.,* 2016). This could have resulted from differences in physico-chemical characteristics of the soil from which the actinomycetes were isolated (Yabe *et al*., 2017).

Biochemical characteristics of actinomycetes are used in their identification. The microorganisms isolated in the current study produced biochemical characteristics that are typical of actinomycetes (Table 3). The results concurred with a previous study that was carried out in Tamilnadu, India (Cholarajan and Vijayakumar, 2016). This may have resulted from the isolated actinomycetes strains been the same (Basha *et al.,* 2017).

On carbon source utilization, the results slightly differed with a previous study (Tebo *et al*., 2015). The difference could have originated from differences in the study area. According to Memon *et al.* (2016) the environment in which actinomycetes are growing in determine their carbon source requirements. In addition Arya and Singh (2016) affirm that different actinomycetes have different carbon source requirements based on their metabolic pathways.

REFERENCES

Ajunwa, O. M., Audu, J. O., Adedeji, B. A., and Ja'afaru, M. I. (2016). Assessment of *in vitro* effects of chlorpyrifos on biomass and antimicrobial production functionality of *Actinomycetes* isolated from soils in Yola, Nigeria. New York Science Journal; 9(6:52-57.

Arya, G. and Singh, G. (2016). Statistical analysis of actinomycetes isolated from the Haridwar region of Uttarakhand (India). Journal of drug delivery and therapeutics; 6(4):31-36.

Ashokvardhan, T. and Satyaprasad , K. (2016). Antibacterial activity of actinomycetes isolated from *Capsicum annuum* L. International journal of pharma and bio sciences; 7(1): 397 – 402.

Atsuko, M. and Takahashi, T. 2017.Endophytic actinomycetes: promising source of novelbioactive compounds. The journal of antibiotics;3:1–6.

Atta, H. M. (2015).Biochemical studies on antibiotic production fromStreptomyces sp.: Taxonomy, fermentation,isolation and biological properties.Journal of Saudi Chemical Society; 19:12–22.

Basha, C. S. and Sambasiva, K. R. S. (2017). An intrinsic assessment of bioactive potentiality of mangroves actinomycetes. Journal of entomology and zoology studies; 5(1): 20-26.

Cassir, N., Rolain, J. M. and Brouqui, P. (2014). "A new strategy; to fight antimicrobial resistance: the revival of old antibiotics.". Frontiers in microbiology;5: 551-560.

Chavan, V., Mulaje S. S. and Mohalkar R. Y. (2013). A review on actinomycetes and their biotechnological application.International journal of pharmaceutical sciences and research; 4(5): 1730-1742.

Cholarajan, A. and Vijayakumar, R. (2016). screening of antibiotic (Staurosporine) producing actinobacteria (*Streptomyces sp.*) from terrestrial environment soil of Thanjavur District, Tamilnadu, India.European journal of biomedical and pharmaceutical sciences; 3(3):480-493.

Chavan, V., Mulaje S. S., Mohalkar R.Y. (2013). A review on actinomycetes and their biotechnological application.International Journal of pharmaceutical sciences and research, 4(5): 1730-1742.

Duddu, M. K. and Guntuku, G. (2016). Isolation, screening and characterization of antibiotic producing actinomycetes from Kapuluppada plastic waste dumping yard, Visakhapatnam.International journal of pharmacy and pharmaceutical sciences; 8 (11): 1-9.

Humera, M., Tumane, P. M. and Durgesh, D. W. (2016). Isolation and Characterization of Anti-Mrsa Producing Actinomycetes from Soil. International journal of recent science research;7(10): 13564-13568.

Ibrahim, S. Y. and Abd El-Salam, M. M (2016). Isolation of anti-fungal agent from a soil inhabitant Streptomyces albaduncus-M51 and its efficacy against osmophilic food spoilage by Saccharomyces cerevisiae. Journal of environmental and occupational science; 5(2): 38-46.

Janaki, T., Nayak, B. K. and Ganesan, T. (2016).Antibacterial activity of soil actinomycetes from themangrove *Avicennia marina*. Journal of pharmacognosy and phytochemistry; 5(1): 267-271.

José, V. L., Suzana, C. S., Katia, A. S., Marcos, A. S. and Claudia, M. M. (2017). Characterization of actinobacteria from the semiarid region, and their antagonistic effect on strains of rhizobia. African journal of biotechnology; 16(11):499-507.

Khasabuli, O. Y. and Kibera, A. N. (2014). Isolation, characterization and primary screening of soil actinomycetes from Kenyatta University arboretum grounds for antibacterial activities. Journal of applied biosciences; 74:6072– 6079.

Kumari, M., Menghani, E.and Mithal, R.(2016).Actinomycetes as Antibiotic Agents: Future generation Bio-factory. International journal of scientific and engineering research; 7(3): 1286- 1296

Manojkumar, S., and Subbaiya, R. (2016). Isolation and Identification of Soil derived Actinomycetes *Nocardiopsis alba.* Research journal of pharmaceutical, biological and chemical sciences; 7(3): 485-493.

Memon, H., Tumane, P. and Wasnik, D. (2016). Isolation and characterization of anti-MRA producing actinomycetes from soil. International journal of recent scientific research; 7(10): 13564-13568.

Modi, S., Chauhan, B., Gahlout, M and Prajapati, H. (2016). Extraction and isolation of melanin from actinomycetes. International journal of drug research and technology; 6(2): 79-86.

Pooja, P., Lekshmi, M. and Jayadev, A. (2016).Isolation and Characterization of Actinomycetes from Mangrove Samples. International journal of science research; 2(9): 1-10.

Prashith, M., Pondet, K., and Kwantep, K. (2015).*In vitro* antimicrobial and antioxidant activities of bioactivecompounds (secondary metabolites) extracted from *Streptomyceslydicus* A2. Science, Technology and arts research journal;3(4): 116-121.

Qais, M. A., Maher, A., Ola, A. A. and Abdullah, Y. A. (2017).New Records of Streptomyces and Non Streptomyces Actinomycetes Isolated from Soils Surrounding Sana'a High Mountain. International journal of research in pharmacy and biosciences; 3(3): 19-31.

Rajan, B. M. and Kannabiran, K. (2014). Extraction and identification of antibacterial secondary metabolites from marine *Streptomyces* sp. VITBRK2. International journal of molecular cell medicine; 3(3): 130-137.

Rotich, M., Magiri, E., Bii, C. and Naomi, M. (2017). Bio-Prospecting for Broad Spectrum Antibiotic Producing Actinomycetes Isolated from Virgin Soils in Kericho County, Kenya. Advances in microbiology; 7:56-70.

Saravana, K., Duraipandiyan, V. and Ignacimuthu, S. (2014). Isolation, screening and partial purification of antimicrobial antibiotics from soil Streptomyces sp. SCA 7. Kaohsiung journal of medical sciences; 30, 435-446.

Siddique, S., Syed, Q., Ahmad, A. and Qureshi, F. A. (2014). Isolation, Characterization and Selection of Avermectin-Producing *Streptomyces avermitilis* Strains from soil samples. Jundishapur journal microbiology. 7(6): e10366.

Slam, M. S., Aktar, M. B., Rahman, M. M. and Main, K. M. (2014). Isolation and characterization of *Streptomyces* spp collected from Bangladeshi soils on the basis of morphological and biochemical studies.International Journal of Current Microbiology *and* Applied *Sciences;* 3(11): 734-742.

Surabhi, T. and Srividya, S. (2016).Lytic actinomycete based extraction of Polyhydroxyalkanoates (PHAs) from *Bacillus megaterium* Ti3. International journal of advanced life sciences; 9(2):227-233.

Tebo, B. M., Davis, R. E., Anitori, R. P., Connell, L. B., Schiffman, P. and H. Staudigel, H. (2015). Microbial communities in dark oligotrophic volcanic ice cave ecosystems of Mt. Erebus, Antarctica. Frontiers of Microbiology; 6:1–14.

Varalakshmi, T., Sekhar, K and Charyulu, P. B. (2014). Taxonomic studies and phylogenetic characterization of potential and pigmented antibiotic producing actinomycetes isolated from rhizosphere soils. International journal of pharmacy and pharmaceutical sciences; 6 (6): 511-519.

Wadetwar, R. N. and Patil, A. T. (2013). Isolation and characterization of Bioactive Actinomycetes from soil in and around Nagpur. International journal of pharmaceutical sciences and research;4(4): 1428-1433.

Wang, X., X. Wang, W. Zhang, *et al.* 2016. Invariant community structure of soil bacteria in subtropical coniferous and broadleaved forests. Scientific reports; 6:19071.

Yabe, S., Sakai, Y., Abe1, K. and Yokota, A. (2017). Diversity of *Ktedonobacteria* with actinomycetes-like morphology in terrestrialenvironments. Microbes environments; 32:(1) 61-70.

Zakalyukina*, Y.*, Biryukov, M. V., Golichenkov,M.V. and Netrusov*, A. I.* (2017). Phenotypic and Phylogenetic Characterization of actinomycetesisolated from *Lasius niger* and *Formica cunicularia* Ants. Moscow University biological sciences bulletin, 72(1) 13–19.

Zhang, X., Chongxi, L., Yuejing, Z., Haiya, W., Shurui, W. Xiangjing, W. Wensheng, X. (2014). Erratum to: Streptosporangium shengliensis sp. nov., a novel actinomycete isolated from a lake sediment. Antonie van Leeuwenhoek; 105:265

Cite this Article: Waithaka PN, Mwaura FB, Wagacha JM and Gathuru EM, 2017. Isolation of Actinomycetes from the Soils of Menengai Crater in Kenya. Greener Journal of Microbiology and Antimicrobials, 3 (2): 008-017. http://doi.org/10.15580/GJMA.2017.2.041817051