BioBacta

Journal of Medical and Life Science
https://jmals.journals.ekb.eg/

Seasonal variation of antibiotic-producing actinomycetes from Menengai crater, Kenya

Paul Njenga Waithaka[1] and Benson Muriuki Githaiga[2]

[1] The Kirinyaga University of Nairobi, School of Pure and Applied Sciences, P. O. Box, 143-10300 Kerugoya, Kenya

[2]Egerton University, Department of Biological Sciences, P. O. Box, 536 Egerton, Kenya

Corresponding author; Paul Njenga Waithaka, Cell; +254 720631404, Email; waithakanj@gmail.com/pnjenga@kyu.ac.ke

DOI: 10.21608/jmals.2023.305819

Abstract

Objectives: The indiscriminate use of antibiotics has led to an upsurge in antibiotic resistance in many parts of the world. This study aimed at determining the seasonal variation of antibiotic-producing actinomycetes from Menengai crater, Kenya.

Methods: Soil samples were collected in February which receives 52mm of rainfall, July (100mm), and May (194mm). Actinomycetes were isolated using serial dilution and spread plate bioassays. The antibiotic property of the isolates against *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumoniae* (ATCC 49617), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus vulgaris* (ATCC 49990) was determined using primary and secondary screening methods. Based on the size of the zone of inhibition and a broad spectrum of activity, four actinomyces coded PAN 25, 41, 75, and 110 were selected for further analysis.

Results: The physicochemical properties of the soil samples did not vary significantly (P=0.42). However, the number of actinomycetes isolated varied significantly (P=0.035) between February, May, and July. The mean zone of inhibition varied between 22±0.3 in PAN 110 to 27±0.3mm in PAN 75. Isolate PAN 110 produced an 829bp fragment, PAN 75 (971bp), PAN 41 (1250bp), and PAN 110 (829bp). The 16S rRNA gene sequence and nucleotide BLAST from NCBI revealed that isolate PAN 110 had 99% similarity with *S. acrimycini* strain K30, PAN 75 (*S. luteogriseus* strain ZG728), PAN 41 (*S. indiaensis*), and PAN 25 (*Streptomyces variabilis*).

Conclusion: Very dry and wet conditions reduced the number of actinomycetes isolated from the soils of the Menengai crater.

Key Words; Characterization, Extraction, Isolation, Molecular, and Samples.

Introduction

Volcanic craters are one of the unique environments on the planet Earth (Fernández-Martínez, 2019). They are unexploited, and inadequately studied (Ziyat *et al.*, 2019). Volcanic craters are usually classified based on the type of rock and formation method. The microbial diversity of volcanic craters has not received the attention they deserve from microbiologists (Majidzadeh *et al.*, 2021). However, there is a likelihood that microorganisms obtained from unexplored craters may belong to new taxonomic units with unique genes that code for the production of novel bioactive compounds (Al-Ansari *et al*., 2019).

Receive Date: 19 April 2023, Accept Date: 23 June 2023, Published: 30 June 2023

The inadequately explored environment such as volcanic craters has a significant potential for exploring new antibiotics (Hamedi *et al.,* 2019). They harbor a variety of microorganisms that lead to increased competition in the environment due to the limited nutrients. As a result, the microorganism produces antimicrobial substances against each other (Kemung *et al.,* 2018).

Among the actinomycetes, the genus *Streptomyces* has produced over 70% of known antibiotics (Pipite *et al.,* 2022). However, some antibiotics have been extracted from rare actinomycetes (non-*Streptomyces* species). In the recent past, antibiotics produced by rare Actinomycetes have increased significantly by 25–30%. This has been attributed to improvements in techniques for the selective isolation of rare Actinomycetes and technologies for genetic characterization (Takahashi and Nakashima, 2018).

The phylum Actinomycetota is one of the largest in Domain Bacteria. It's made of six classes, 32 orders, and 73 families. All Actinomycetes are filamentous unicellular Gram-positive bacteria. They possess a high GC content in their genome and are omnipresent in both terrestrial and aquatic environments (Parmar *et al.,* 2016). When growing on a culture medium, they form the substrate and aerial mycelium (Park *et al.,* 2014). They reproduce by binary fission, fragmentation, or by producing spores that are also known as conidia (Genilloud, 2017). Among the Actinomycetes thermophilic species are growing under 45–65°C, acidophilic species (pH 3.5–6.5), halophilic species (0.5–4 M salt), endophytic species, symbionts, and endosymbionts (Amsaveni *et al.,* 2015).

Actinomycetes produce some bioactive compounds. Some produce enzyme inhibitors involved in the bioconversion, biocontrol, and degradation of hydrocarbons (Rangseekaew and Pathom-aree, 2019).). Significantly, actinomycetes produce compounds that provide defenses against pathogenic microorganisms collectively known as antibiotics (Tandal *et al.,* 2018).

Although soils have been screened to produce novel bioactive compounds with many agricultural, pharmaceutical, and industrial applications, only a small portion of the earth's surface has been sampled and studied (Vishwanatha *et al.,* 2017). A major challenge is the isolation of actinomycetes that produce known antibiotics. However, researchers have reverted to screening for novel and biotechnologically exploitable microorganisms that yield new and diverse antibiotic activity (Tan *et al.,* 2015). This has been made possible by exploiting previously unstudied geographical regions (Maiti *et al.,* 2020). Therefore, current isolation strategies for actinomycetes capable of producing novel antibiotics require new sample sources from uncommon and unexplored habitats. In this regard, the current study was designed to explore the seasonal variation in antibiotic-producing actinomycetes from the Menengai crater.

Materials and methods

The study area

Menengai crater which lies North of Lake Nakuru. It has a height of 2,278 m above sea level and is a product of a volcanic eruption that occurred about 200,000 years ago. The crater is dormant with a high-temperature geothermal resource indicated by steaming grounds at a temperature of 88^{o}C. The eruption yielded a large hole in the crust, called a caldera. The caldera occupies an area of 90 km^2 and a diameter of 12 km. The caldera is the second largest in Africa after Ngorongoro in Tanzania (Omenda *et al*., 2000).

Soil sample collection

Soil samples were separately collected from the top 5cm using a sterile spatula in February with an average rainfall of 52mm and an average temperature of 27.22^{o}C, May (average rainfall of 194mm; average temperature of 25.24^{o}C) and July (average rainfall of 100mm; average temperature 23.23^{o}C). The samples for each month were mixed to make a composite sample. The composite samples were placed in new khaki bags and transported to the Department of

Biological Sciences Laboratories, Egerton University for analysis.

Physico-chemical analysis

Each soil sample was suspended in distilled water. Soil pH was determined according to the procedure described by Guo *et al*. (2015). The pH meter was standardized using a standard solution provided. Immediately before immersing the electrode(s) into the sample, the sample well was stirred with a glass rod. The electrode(s) were placed into the soil slurry solution and the beaker was gently turned to make good contact between the solution and the electrode(s). Organic carbon was determined using the wet oxidation method (Singh *et al*., 2015).

The Kjeldahl method (Khursheed *et al*., 2014) was used to determine total nitrogen. Phosphorus was obtained by Bray and Kurtz-1 method and determined spectrophotometrically (Osakwe, 2014). Potassium and sodium content in the soil was determined by using turbidimetric methods; calcium by titration with standard KMnO4 solution, and magnesium by precipitation in an alkaline medium as magnesium ammonium phosphate (Salam and Rana 2014). Manganese was analyzed by Atomic Absorption Spectrophotometer using GBC Avanta version 1.31 by flame Automization (Egejuru *et al*., 2014)

Isolation of actinomycetes

Actinomycetes were isolated using the serial dilution plate method. Briefly, 1 g of each soil sample was weighed and suspended in 9 mL of distilled water to make 10^{-1} to 10^{-5} dilutions. Using a micropipette, 0.1 mL aliquots of each concentration were dispensed into sterile Petri dishes with 15 mL of sterile Luria Bertani (M1) medium (starch 10 g, Peptone 2.0 g, yeast Extract 4.0 g, Agar 18.0 g, distilled water 1000 ml, pH; 7.0 ± 0.1), spread using an L shaped glass rod and incubated at 25^{o}C for 5d (Waithaka *et al.*, 2018). A colony counter was used to determine the actinomycetes colonies and microbial load calculated in CFU/mL. To obtain pure cultures, the microbes were subcultured by streaking on M1 media. The pure isolates were stored in slant bottles for characterizations using colony morphology and biochemical characteristics (Yang *et al*., 2015).

Primary and secondary screening of actinomycetes for antagonism against test pathogens

Staphylococcus aureus (ATCC 25923), *Streptococcus pneumoniae* (ATCC 49617), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus vulgaris* (ATCC 49990) were used as test organisms. Primary screening for antagonism was carried out using the perpendicular method while secondary screening was carried out using the disk diffusion technique (Devadass *et al.*, 2016). Four actinomycetes abbreviated PAN 25, PAN 41, PAN 75, and PAN 110 were selected for further analyses based on a broad spectrum of activity and the size of the zone of inhibition. The selected antagonistic antimicrobial strains were separately inoculated into 3L of M1 broth, and incubated at 28°C in a shaker (200 rpm) for seven days. Extraction of antibiotics was carried out using ethyl acetate and the extracts were tested against the test pathogens using the Kirby Bauer disc diffusion method (Tyagi *et al.*, 2014).

Molecular characterization of the selected actinomycetes

Extraction of the actinomycetes DNA

The four selected actinomycetes isolates were grown in 10mL M1 medium at 28°C for 48h (Prashith *et al*., 2012). Actinomycetes were harvested from 1.5mL of culture by centrifugation at 7500x g for 10 min. The cell pellets obtained were washed with 500µl of 10mMTE buffer (10 mM Tris–HCl, pH 7.7, and 1 mM EDTA). The washed pellets were re-suspended in 200µl lysis buffer (1M Tris-HCl, pH 8.0, 0.5 M EDTA, 5M NaCl, and 10% SDS) and incubated at 60°C for 30min. Subsequently, 8µl of proteinase K (10mg/ml) was separately added and incubated at 55°C for 1h. To these mixtures, 200µl of phenol: chloroform: isoamyl alcohol (25:24:01) was added and vortexed thoroughly for 4min. Each mixture was centrifuged at 10,000-x g

for 10min. The aqueous phases were transferred to new tubes and the DNA precipitated with 2 volumes of 95% ethanol. The DNA pellets obtained by centrifugation at 10,000 x g for 5 min were resuspended in 40µl of TE buffer, pH 8.0. The purity of DNA was each confirmed by biophotometer (Eppendorf, India) and agarose gel (0.8%) electrophoresis (Zhang *et al*., 2019).

PCR amplification of the 16S rRNA gene

The 16S rRNA gene of the selected actinomycetes strains was amplified between positions 8 to 1492 using the forward and reverse primers (Scorzoni *et al*., 2017). PCR reactions mixture (20µl) having 2µl of 10x Taq buffer (Sigma), 200 mM dNTP, 0.2 mM of each primer (Sigma, USA), 3mM $MgCl_2$, 2.5U Taq DNA polymerase (Sigma), and 20ng of template DNA was used. The cycling conditions were; initial denaturation at 95°C for 5min, followed by 35 cycles of denaturation at 95°C for 30sec, annealing at 63°C for 30sec, extension at 72°C for 30 sec, and final extension at 72°C for 10min. The PCR products were pooled and purified on QIA quick PCR purification columns (Qiagen). Approximately 1500 bp of PCR products were separately sequenced using ABI DNA sequencer, Applied Biosystems (Bioserve Biotechnologies (India) Pvt Ltd, Hyderabad) (Hathaway *et al*., 2014).

Sequence Similarities and phylogenetic analysis

Nucleotide sequences of the 16S rRNA of the selected actinomycete isolates were determined and compared for similarity level with the reference species of actinomycetes contained in the genomic database bank. The BLAST program (http://www.ncbi.nlm.nih.gov/blast) was used in assessing the degree of DNA similarity. Multiple sequence alignment and molecular phylogeny were evaluated using BioEdit software (Jayshree *et al*., 2016). The phylogenetic tree was constructed using the PHYLIP software (http://evolution.genetics.washington.edu/phylip/software.html).

Data analysis

Data were presented using tables. All the data analysis was carried out using Statistical Package of Social Sciences Software (SPSS) software version 17.0 software and a Microsoft 2010 spreadsheet. One-way ANOVA was used in comparing the means.

RESULTS

Physico-chemical characteristics of soil from Menengai crater

The pH ranged from 5.01 in May to 6.62 in February, phosphorus from 4.15 in May to 15.53 in February, potassium from 0.35 in May to 1.50 in February, Nitrogen from 0.10 in May to 0.29 in February, calcium from 3.45 in May to 16.56 in February, magnesium from 2.40 in May to 2.65 in February, manganese from 0.31 in May to 0.51 in February, sodium from 0.02 in May to 0.15 in February, carbon from 1.05 in May to 2.50 in July (Table 1). The Ca: Mg ratio varied from 15.61 in February to 20.14 in May. The physicochemical properties of the soil samples did not vary significantly (P=0.42).

Table 1: Physico-chemical properties of soil samples from Menengai crater

Physico-chemical properties of soil	February	May	July	Recommended Levels	
				Low	High
pH	6.62	5.01	5.62	5.50	7.00
Phosphorus	15.53	4.15	15.00	20.00	100.00
Potassium	1.50	0.35	1.32	0.20	1.50
Nitrogen	0.29	0.10	0.20	0.20	0.80
Calcium	16.56	3.45	15.34	2.00	10.00
Magnesium	2.65	2.40	2.59	1.00	3.00
Manganese	0.51	0.31	0.45	0.10	1.00
Sodium	0.15	0.02	0.10	0.10	2.00
Carbon	2.40	1.05	2.50	2.00	5.00
Ca: Mg ratio	15.61	20.14	16.00	4.00	7.00

Actinomycetes from Menengai crater

The number of actinomycetes isolates at a dilution of 10^{-1} varied from $3.60x10^2$ to $2.22x10^4$ CFU/g, 10^{-2} ($2.32x10^2$-$1.43x10^4$ CFU/g), 10^{-3} ($2.00x10^2$-$4.15x10^3$ CFU/g), 10^{-4} ($1.51x10^1$- $3.34x10^3$ CFU/g and 10^{-5} ($1.40x10^1$-$2.20x10^2$ CFU/g) (Table 2). The mean actinomycetes isolates ranged from $2.17x10^2$ CFU/g in February to $6.67x10^3$ CFU/g in July. The number of actinomycetes isolated varied significantly (P=0.035) between February, May, and July.

Table 2: Number (CFU/g) of actinomycetes isolated from Menengai crater in February, May, and July.

Dilution factor	February	May	July
10^{-1}	$3.60x10^2$	$5.00x10^2$	$2.22x10^4$
10^{-2}	$2.32x10^2$	$3.70x10^2$	$1.43x10^4$
10^{-3}	$2.00x10^2$	$2.19x10^2$	$4.15x10^3$
10^{-4}	$1.51x10^1$	$1.3x10^1$	$3.34x10^3$
10^{-5}	$1.40x10^1$	$1.10x10^1$	$2.20x10^2$
Mean	**$2.17x10^2$**	**$2.66x10^2$**	**$6.67x10^3$**

Antibiotics producing actinomycetes from Menengai Crater

The zone of inhibition for PAN 25 ranged between 20±0.3 to 28±0.2, PAN 41 (22±0.2-25±0.2mm), PAN 75 (25±0.3-30±0.3mm) and 110 (21±0.2-23±0.3mm) (Table 3). The mean zone of inhibition varied between 22±0.3 in PAN 110 to 27±0.3mm in PAN 75. However, there was no significant difference in the zones of inhibition between the isolates (P=0.496).

Table 3: Zones of inhibition (mm) of the test pathogens by the actinomycetes isolates

Isolate	*S. au*	*S. p*	*E. fa*	*E. co*	*P.ae*	*P.vu*	Mean
PAN 25	24±0.2	27±0.3	26±0.2	28±0.2	20±0.3	22±0.2	24±0.2
PAN 41	23±0.2	22±0.2	24±0.2	25±0.2	22±0.2	23±0.2	23±0.2
PAN 75	26±0.3	30±0.3	27±0.3	29±0.2	26±0.3	25±0.3	27±0.3
PAN 110	23±0.3	22±0.2	23±0.3	21±0.2	22±0.3	23±0.3	22±0.3

S. au; Staphylococcus aureus, S. p; Streptococcus pneumoniae, E. fa; Enterococcus faecalis, E. co; Escherichia coli, P. ae; Pseudomonas aeruginosa and *P. vu; Proteus vulgaris.*

16S rRNA and polymerase chain reaction

Isolate PAN 110 produced an 829bp fragment, PAN 75 (971bp), PAN 41 (1250bp), and PAN 110 (829bp) (Figure 1). Specific primers were used in polymerase chain reaction (PCR) to amplify the genes.

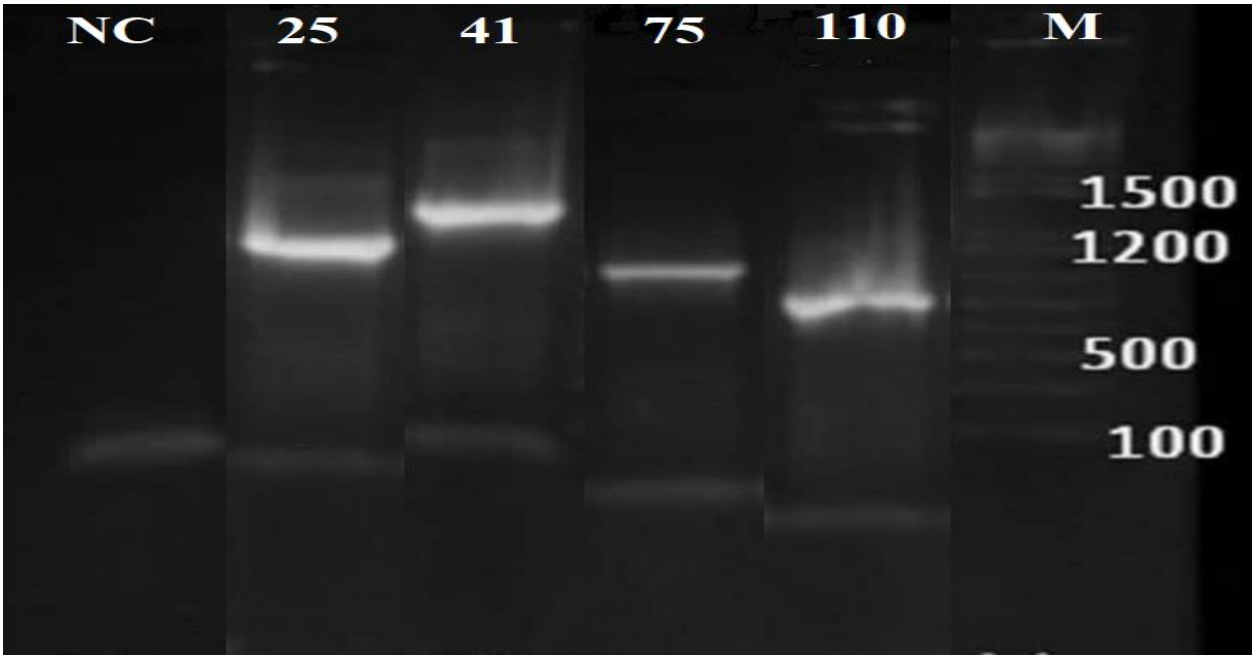

Figure 1: Amplifications of 16S rDNA of PAN 110, 75, 41, and 25; NC, negative control; M, molecular ladder.

Sequence similarities and phylogenetic analysis of the selected actinomycetes

The 16S rRNA gene sequence and nucleotide BLAST from NCBI revealed that isolate PAN 110 had 99% similarity with *Streptomyces acrimycini* strain K30, PAN 75 (*S. luteogriseus* strain ZG728), PAN 41 (*S. indiaensis*) and PAN 25 (*S. variabilis*) (Figure 2).

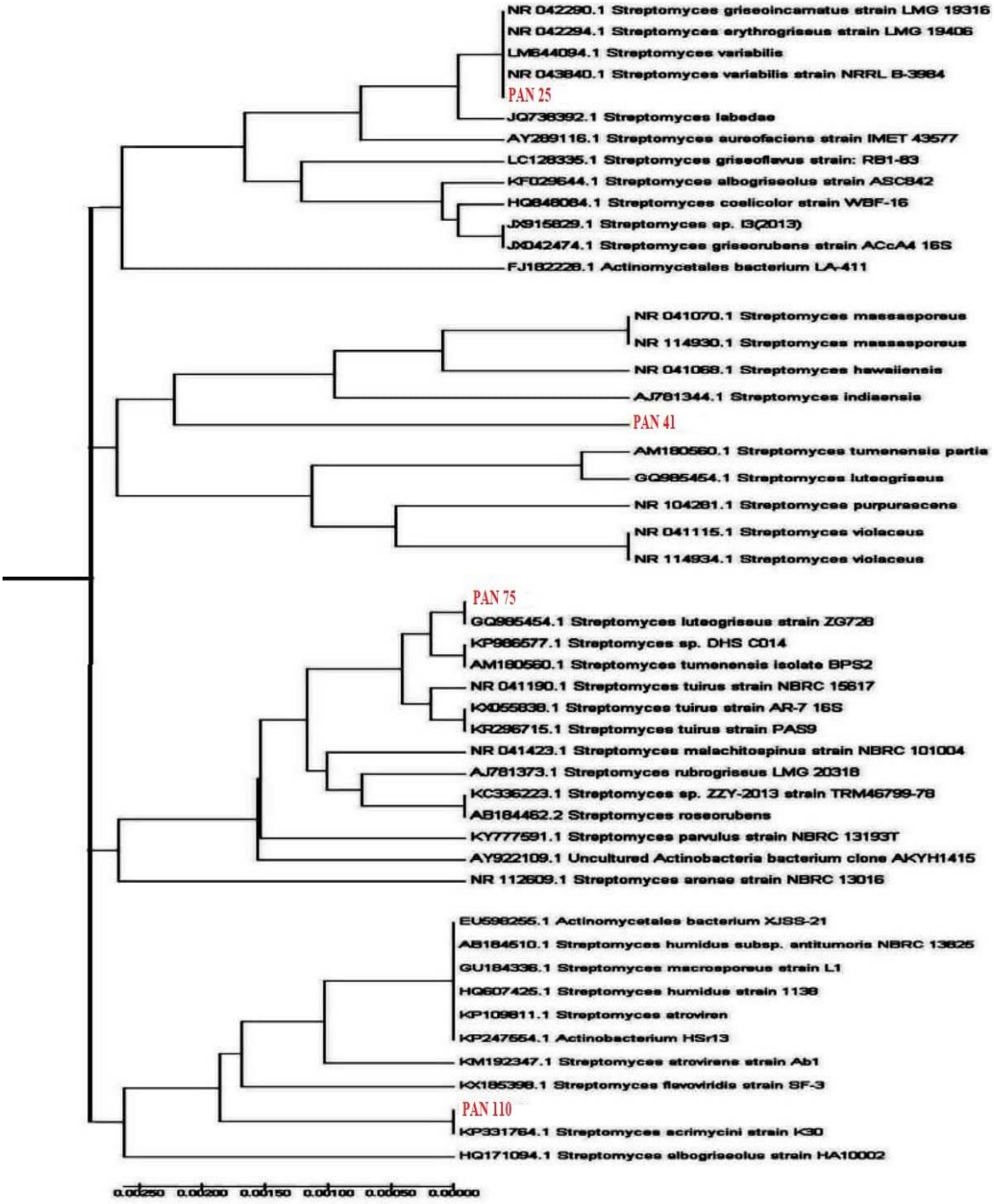

Figure 2: Phylogenetic tree of actinomycete isolates PAN 110, PAN 75, PAN 41, and PAN 25 constructed using the neighbor-joining technique.

Discussion

The soils of the Menengai crater were formed from the solidification of magma after a volcanic eruption. The chemical composition of the magma determined the physicochemical characteristics of the soils. The results of this study indicated that the physico-chemical properties of the soil were high in February the driest month, moderate in July, and lowest in May which had the highest amount of rainfall. The findings concurred with those of a previous study carried out by Hueso *et al.* (2012). Lagomarsino *et al.* (2012) explained that during the rainy seasons, a lot of soil minerals dissolve in water and are carried deep into the soil horizons which is contrary to what happens in the dry season.

The number of actinomycetes recovered from the soil samples in this study was low in February and May and high in July. February was the driest month in the study site while May received the highest amount of rainfall. A moderate amount of rainfall was received in July when the highest number of actinomycetes were isolated. Giordano (2020), explained that too dry or wet soil conditions lead to a low number of actinomycetes isolates. Further, Borowik and Wyszkowska (2016) ascertained that very low water activity during the dry months reduces the multiplication of actinomycetes. Besides Giacometti *et al.* (2013) maintained that running floods experienced during heavy downpours carry with them actinomycetes leading to low counts.

Among the selected actinomycetes PAN 75 produced the biggest mean zone of inhibition when compared with PAN 110, PAN 41, and PAN 25. The zones of inhibition observed were bigger than in previous studies (Adam *et al.,* 2018; Belyagoubi *et al.,* 2018; Long *et al.*, 2019). This may be attributed to differences in the species of actinomycetes isolated (Matroodi *et al.,* 2020). The differences in soil characteristics from which the actinomycetes were isolated influence the types of antibiotics produced by actinomycetes (Iquebal *et al.,* 2021). In addition, environmental factors such as temperature influence the growth and production of antibiotics by actinomycetes (Ameerah *et al*., 2015).

Currently, the use of 16S rRNA in identifying actinomycetes is more popular than the use of traditional methods (Hug *et al.,* 2018). In addition, the use of molecular techniques for the identification of actinomycetes is quick and has increased reliability (Hifnawy *et al.,* 2020). Almalki, 2020, asserted that 16S rRNA sequences cannot be shared making the technique one of the most reliable in the identification of microorganisms. The results of the present study on molecular characterization of the selected actinomycetes differed from those of previous studies (Hamedi *et al.,* 2019; Eid *et al.,* 2020; Hifnawy *et al.,* 2020; Bontemps *et al.,* 2021). Differences in the study areas which expose the actinomycetes to varying ambient conditions leading to a change of genetic constitution in actinomycetes may have been a contributing factor (Hahn *et al.,* 2019).

Conclusion

The physicochemical characteristics of soils from the Menengai crater were typical of soils from volcanic craters. Soil samples from the Menengai crater had antibiotic-producing actinomycetes. Very dry and wet conditions reduced the number of actinomycetes isolated from the soils of the Menengai crater. The actinomycetes isolated from the soil samples belonged to the genus *Streptomyces*.

Recommendations

There is a need to carry out similar studies in other volcanic areas in Kenya. The antibiotics from the selected actinomycetes isolates need to be characterized. In addition, there is a need to test the antibiotics against other pathogens.

Conflict of interest

No conflict of interest is to be declared.

Financial disclosure: No financial support was received for this study

Author Disclosure Statement: No competing financial interests exist.

Ethical Approval: Ethical approval was not required.

References

1. Adam, D., Maciejewska, M., Naômé, A., Martinet, L., Coppieters, W. and Karim, L. 2018. Isolation, characterization, and anti-bacterial activity of hard-to-culture actinobacteria from cave moonmilk deposits. Antibiot. 7,28-37 doi: 10.3390/antibiotics7020028
2. Al-Ansari, M., Alkubaisi, N., Vijayaragavan, P. and Murugan K. 2019. Antimicrobial potential of *Streptomyces* sp. to the Gram positive and Gram negative pathogens. J. Infect. Public Health. 12,861–866.
3. Almalki, M. A. 2020. Isolation and characterization of polyketide drug molecule from Streptomyces species with antimicrobial activity against clinical pathogens. J. Infect. Public Health, 13:125–130. doi: 10.1016/j.jiph.2019.07.002
4. Ameerah, H. A., Suhaidi, A., Sharifah, A. and Syed, M. 2015. Identification and optimal growth conditions of actinomycetes isolated from Mangrove environment. Malaysian J. Anal. Sci. 19(4),904 – 10.
5. Amsaveni, R., Sureshkumar M. and Vivekanandhan G. 2015. Screening and isolation of pigment producing Actinomycetes from soil samples. Int. J. Biosci. Nanosci. 2(2),24-28.
6. Belyagoubi, L., Nabila, B. B., Valme, J., Joëlle, D., Sandrine, L. and Fatima, D. 2018. Antimicrobial activities of culturable microorganisms (actinomycetes and fungi) isolated from Chaabe cave. Int. J. Speleol. 47,189–199. doi: 10.5038/1827-806X.47.2.2148
7. Bontemps, Z., Alonso, L., Pommier, T., Hugoni, M., and Moënne-Loccoz, Y. 2021. Microbial ecology of tourist Paleolithic caves. Sci. Total Environ. 816,151492. doi: 10.1016/j.scitotenv.2021.151492
8. Borowik A. and Wyszkowska J. 2016. Soil moisture as a factor affecting the microbiological and biochemical activity of soil. Plant Soil Environ. 62(6),250-255.
9. Devadass, B. J., Paulraj, M. G., Ignacimuthu, S., Theoder, P. A. S. and Dhabi, N. A. A. 2016. Antimicrobial activity of soil actinomycetes isolated from Western Ghats in Tamil Nadu, India. J. Bacteriol. Mycol. 3(2),00059. https://doi.org/10.15406/jbmoa.2016.03.00059
10. Egejuru, O., Emmanuel, I., Akubugwo, I. and Victor, C. 2014. Evaluation of physicochemical properties, microbial loads and enzymes activity studies of agrochemicals on the Imo-River basin farm in Imo State Nigeria. Int. J. Curr. Microbiol. Appl. Sci. 3(2),776-786.
11. Eid, A. M., Fouda, A., Niedbała, G., Hassan, S. E.-D., Salem, S. S. and Abdo, A. M. F. 2020. Endophytic *Streptomyces laurentii* mediated Green synthesis of ag-NPs with antibacterial and anticancer properties for developing functional textile fabric properties. Antibiot. 9:641-650. doi: 10.3390/antibiotics9100641
12. Fernández-Martínez, L.T. 2019. Actinomycetes as nature's pharmacists. Natural products and drug discovery. Actinomycetes as nature's pharmacists. Microbiol. Societ. 23,234-244.
13. Genilloud, O. 2017. Actinomycetes: still a source of novel antibiotics. Nat. Prod. Rep. 34(10),1203-1232.
14. Giacometti, C., Demyan, M. S., Cavani, L., Marzadori, C., Ciavatta, C. and Kandeler, E. 2013. Chemical and microbiological soil quality indicators and their potential to differentiate fertilization regimes in temperate agroecosystems. Appl. Soil Ecol. 64,32-48.

15. Giordano, D. 2020. Bioactive Molecules from Extreme Environments. Mar. Drugs; 18:640. doi: 10.3390/md18120640.
16. Guo, X., Liu, N., Li, X., Ding, Y., Shang, F., Gao, Y., Ruan, J. and Huang, Y. 2015. Red Soils harbor diverse culturable Actinomycetes that are promising sources of novel secondary metabolites. Appl. Environ. Microbiol. 81(9), 3086-103.
17. Hahn, M. W., Koll, U., and Schmidt, J. 2019. "Isolation and cultivation of bacteria. Adv. Environ. Microbiol. 7,313–321.
18. Hamedi, J., Kafshnouchi, M., and Ranjbaran, M. 2019. A study on Actinobacterial diversity of Hampoeil cave and screening of their biological activities. Saudi J. Biol. Sci. 26,1587–1595. doi: 10.1016/j.sjbs.2018.10.010
19. Hathaway, J. J. M., Garcia, M. G. and Moya, M. 2014. Comparison of bacterial diversity in Azorean and Hawaiian lava cave microbial mats. Geomicrobiol. J. 31,205–20.
20. Hifnawy, M. S., Fouda, M. M., Sayed, A. M., Mohammed, R., Hassan, H. M. and AbouZid, S. F. 2020. The genus *Micromonospora* as a model microorganism for bioactive natural product discovery. RSC Adv. 10:20939–20959. doi: 10.1039/D0RA04025H
21. Hueso, S., García, C. and Hernández, T. 2012. Severe drought conditions modify the microbial community structure, size, and activity in amended and unlamented soils. Soil Biol. Biochem. 50,167–173.
22. Hug, J. J., Bader, C. D., Remškar, M., Cirnski, K., and Müller, R. 2018. Concepts and methods to access novel antibiotics from Actinomycetes. Antibiot. 7,44-54. doi: 10.3390/antibiotics7020044.
23. Iquebal, M. A., Passari, A. K., Jagannadham, J., Ahmad, F., Leo, V. V. and Singh, G. 2021. Microbiome of Pukzing cave in India shows high antimicrobial activity against plant and animal pathogens. Genomics. 113,4098–4108. doi: 10.1016/j.ygeno.2021.10.004
24. Jayshree, D. P., Juliann, D., and Huda, M. 2016. Discovery of antibiotic biomolecule(s): a multidisciplinary approach. Int. J. Biochem. Res. 5(7),112-118.
25. Kemung, H. M., Tan, L. T. H., Khan, T. M., Chan, K. G., Pusparajah, P. and Goh B. H. 2018. *Streptomyces* as a prominent resource of future anti-MRSA drugs. Front. Microbiol. 9,1-26.
26. Khursheed, A. W., Rajni, Y., Shivom, S. and Krishan, K. U. 2014. Comparative study of physicochemical properties and fertility of soils in Gwalior, Madhya. Pradesh. World J. Agric. Sci. 10(2),48-56.
27. Lagomarsino, A., Grego, S. and Kandeler, E. 2012. Soil organic carbon distribution drives microbial activity and functional diversity in particle and aggregate-size fractions. Pedobiologia. 55,101–110.
28. Long, Y., Jiang, J., Hu, X., Zhou, J., Hu, J., and Zhou, S. 2019. Actinobacterial community in Shuanghe cave using culture-dependent and-independent approaches. World J. Microbiol. Biotechnol. 35,153-163. doi: 10.1007/s11274-019-2713.
29. Maiti, P. K., Das, S., Sahoo P. and Mandal, S. 2020. *Streptomyces* sp. SM01 isolated from Indian soil produces a novel antibiotic picolinamycin effective against multi-drug resistant bacterial strains. Sci. Rep. 10,123-133.
30. Majidzadeh, M., Heidarieh, P. and Fatahi-Bafghi, M. 2021. Antimicrobial activity of *Actinobacteria* isolated from dry land soil in Yazd, Iran. Mol Biol. Rep. 48(1),121-133.
31. Matroodi, S., Siitonen, V., Baral, B., Yamada, K., Akhgari, A., and Metsa-Ketela, M. 2020.

Genotyping-guided discovery of Persiamycin a from sponge-associated halophilic *Streptomonospora* sp. PA3. Front. Microbiol. 11:1237. doi: 10.3389/fmicb.2020.01237

32. Omenda, P. A., Opondo, K., Lagat, J., Mungania, J., Mariita, N., Onacha, S., Simiyu, S., Wetang'ula, G. and Osakwe SA. 2014. Evaluation of physicochemical characteristics of soils in the flood disaster affected areas of Isoko Region of Delta State, Nigeria. IOSR J. Appl. Chem. 7(5),24-31.
33. Ouma, P. 2000. Ranking of geothermal prospects in the Kenya rift. Kenya Electricity Generating Company Limited, internal report, pp 121.
34. Park, H. B., Lee, J. K., Lee, K. R. and Kwon, H. C. 2014. Angumycinones A and B, two new angucyclic quinones from *Streptomyces* sp. KMC004 isolated from acidic mine drainage. Tetrahedron. 55,63-73.
35. Parmar, R. S., Singh, C., Jadon, P., Bhadauriya, G. and Kumar, A. 2016. Exploration of pigment producing Actinomycetes, isolated from Madhya Pradesh region of India. Int. J. High. Educ. 6(2),961-966.
36. Pipite, A., Lockhart, P. J., McLenachan, P. A., Christi, K., Kumar, D., Prasad, S. and Subramani, R. 2022. Isolation, antibacterial screening, and identification of bioactive cave dwelling bacteria in Fiji. Front. Microbiol. 13,1012867.
37. Rangseekaew, P. and Pathom-aree, W. 2019. Cave Actinobacteria as producers of bioactive metabolites. Front. Microbiol. 10:387-397.
38. Salam, M. D. and Rana, S. 2014. Antimicrobial potential of Actinomycetes isolated from soil samples of Punjab. India J. Microbiol. Exp. 1(2),3-10.
39. Singh, L. S., Sharma, H. and Talukdar, N. C. 2014. Production of potent antimicrobial agent by actinomycete, *Streptomyces sannanensis*stra in SU118 isolated from phoomdi in Loktak Lake of Manipur, India. BMC Microbiol. 14: 278-90. https://doi.org/10.1186/s12866-014-0278-3
40. Takahashi, Y. and Nakashima, T. 2018. Actinomycetes, an inexhaustible source of naturally occurring antibiotics. Antibiot. (3),1-11.
41. Tan, L. T., Ser, H. L., Yin, W. F., Chan, K. G., Lee, L. H. and Goh B. H. 2015. Investigation of antioxidative and anticancer potentials of *Streptomyces* sp. MUM256 isolated from Malaysia mangrove soil. Front. Microbiol. 6,1316-1326.
42. Tandal, A., Khandagale, M., Palaskar R. and Kulkarni, S. 2018. Isolation of pigment producing actinomycetes from soil and screening their antimicrobial activity against different microbial isolates. Int. J. Curr. Res. Life Sci. 7(6),2397-2402
43. Tyagi, J., Bhatnagar, T. and Pandey, F. K. 2014. Isolation and Characterization of Actinomycetes from soil and screening their Antibacterial Activities against different microbial isolates. Int. J. Life Sci. Res. 2(4),101-5.
44. Vishwanatha, B. T., Girish, B. K., Padmashri, S. B., Chethan, J. D. and Sreenivasa, N. 2017. Isolation, identification, and characterization of *Streptomyces* sp. SN-2. Biosci. Biotechnol. Res. Asia. 14(4),1401-1407.
45. Waithaka, N. P., Mwaura, B. F., Wagacha, M. J., Gathuru, M. E. and Githaiga, M. B. 2018. Sensitivity testing, yield, and stability of antimicrobial metabolites obtained from soils of Menengai crater in Kenya. Biomed. Sci. Today. 3, e16.
46. Yang, J., Li, X. Y., Huang, L. Q., and Jiang, H. C. 2015. Actinobacterial diversity in the sediments of five cold springs on the qinghai-

tibet plateau. Front. Microbiol. 6,1345. doi: 10.3389/Fmicb.2015.01345

47. Zhang, B., Wu, X., Tai, X., Sun, L., Wu, M., Zhang, W., Chen X., Zhang, G., Chen, T., Liu, G. and Dyson, P. 2019. Variation in actinobacterial community composition and potential function in different soil ecosystems belonging to the Arid Heihe River Basin of Northwest China. Front. Microbiol. 10,2209. doi: 10.3389/fmicb.2019.02209
48. Ziyat, A., Goddfellow, M., Nurgozhina A., Sergazy S. and Nurgaziev M. 2019. Novel actinobacterial diversity in Kazakhstan deserts soils as a source of new drug leads. Journal of Microbiology, Biotechnology and Food Sci. 8(4):1057-1065.