Advances in Bioresearch
Adv. Biores., Vol 16 (6) November 2025: 116-123
©2025 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.6.116123

Advances in Bioresearch

ORIGINAL ARTICLE

Determination of antibiotic production by selected actinomycetes from Menengai crater in Kenya using liquid and solid-state fermentation

Paul Njenga Waithaka[1] and Benson Muriuki Githaiga[2]
[1]Kirinyaga University, 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
Email: waithakanj@gmail.com, pnjenga@kyu.ac.ke

ABSTRACT
The evolution of pathogens into resistant strains has reduced the effectiveness of most antibiotics in the market today. This calls for discovery of new drugs which requires variations in the methods of culturing antibiotic producing actinomycetes. This study aimed at isolating actinomycetes from the volcanic soils of Menengai crater and comparing liquid and solid state fermentation of selected actinomycetes in antibiotic production. Soil samples were collected from randomly selected sampling points from Menengai crater. Actinomycetes were isolated using starch casein, Luria Bertani and starch nitrate agar media. The test for antibiotic property of the isolated actinomycetes was done against Staphylococcus aureus (ATCC 25923), Streptococcus pneumoniae (ATCC 49617), Pseudomonas aeruginosa (ATCC 27853), Proteus vulgaris (ATCC 49990), Candida albicans (ATCC 10231), and Fusarium oxysporum (ATCC 16608. Four actinomycetes coded PAN 15, PAN 42, PAN 70 and PAN 100 were selected based on size of the zone of inhibition and broad spectrum of activity. Liquid and solid fermentation was used in producing antibiotics from the selected actionmycetes. Extraction of antibiotics was carried out using ethyl acetate and the susceptibility test done against the selected pathogens using Kirby Bauer disc diffusion test. Cytotoxicity of the antibiotics was carried out using brine shrimp lethality bioassay. There was no significant difference in the number of actinomycetes isolated using starch casein, Luria Bertani and starch nitrate agar media (P= 0.34). In addition, there was no significant difference in the zones of inhibition between the isolates (P=0.00071). The yield of the crude antibiotic extract between liquid and solid-state fermentation varied significantly (P = 0.0056). The LC_{50} values ranged from 2. 68±0.27 µg/ml in PAN 15 to 4.97±0.11µg/ml in PAN 100. Solid state fermentation produced high amounts of antibiotics than liquid fermentation. There is need to characterize the antibiotics produced by the selected actinomycetes.
Keywords: *Actinomycetes, antibiotic, extraction, fermentation and isolation.*

Received 29.09.2025 Revised 20.10.2025 Accepted 25.11.2025

How to cite this article:
Paul Njenga W and Benson Muriuki G. Determination of antibiotic production by selected actinomycetes from Menengai crater in Kenya using liquid and solid-state fermentation. Adv. Biores., Vol 16 (6) November 2025: 116-123.

INTRODUCTION

The development of resistant pathogens, evolution of new diseases coupled with the toxicity of some drugs in the market today demands for search of new antibiotics [1]. As much as good progress has been made in biosynthesis of biomolecules, nature presents the richest and the most versatile source of new drug leads [2].

Actinomycetes are the largest producers of antibiotics in the world today [3]. The antibiotics are diverse in structure and usage [4]. Streptomyces spp. has produced over 70% of the currently known antibiotics [5]. In recent years, the discovery of novel antibiotics has greatly declined due to exhaustion of common sources of actinomycetes [6]. Different environments present varying growth conditions to actinomycetes thus influencing the amounts and types of metabolites to be synthesized [7]. Therefore, if novel metabolites are to be found, unique environments such as Menengai crater needs to be explored [8]. Many attempts to isolates actinomycetes from un- and under-explored soil habitats have been made [9].

Previous studies are pointing at extremophilic actinomycetes as the most promising source for antibiotics. This has been attributed to their unique community structure and diverse metabolic pathways [10]. However, little is known and documented about the diversity and the antibiotic potential of actinomycetes from volcanic craters [11]. In isolation of actinomycetes, different media have been suggested [12]. Among them is yeast malt extract agar, tyrosine agar, starch casein agar, glycerol asparagine agar, starch yeast extract agar and glycerol yeast extract agar. The composition of growth media is important for growth and production of antibiotics by actinomycetes [13]. Solid-state fermentation refers to the growth of microorganisms on solid substrates without the presence of free liquid between substrate particles [14]. There are two types of solid matrices. Natural solid materials such as foodstuffs and agricultural by-products which act as the main source of nutrients are one category of the matrices [15]. The second category constitute an inert solid support supplemented with nutrients for microbial growth [16]. These solid materials serve as a physical support for growth through where metabolites can be released by the actinomycetes [17]. This study aimed at isolating actinomycetes from Menengai crater using different media and determining growth and antibiotic production by the isolates under liquid and solid-state fermentation.

MATERIAL AND METHODS

Description of the study area

The study was carried out in Menengai crater that rises to a height of 2,278m above sea level. The crater is a product of a volcanic eruption that occurred about 200,000 years ago. Menengai crater is a dormant volcano which has high temperature geothermal resource at a temperature of around 88^{o}C. After the eruption, the sides of the volcanic crater collapsed inwards forming a large hole in the crust, called a caldera [18].

Soil sampling

Soil samples were collected at a depth of 5–10 cm using a soil auger from 16 randomly selected sampling points and mixed to make a composite sample. The composite sample was transported to the Department of Biological Sciences laboratories in Egerton University. The sample was heat treated at 55°C in a hot air oven to kill vegetative microorganism. The sample was stored at 4^{o}C awaiting further processing and isolation of actinomycetes [19].

Isolation of actinomycetes

The composite sample was sieved using 250µm pore size sieve. Briefly, 2g of the composite sample was placed on aluminium foils and heat treated at 120^{o}C using a hot air oven (Heidolph Laorota, 4001, Buchi Vacuum Conroller V-805) for 1h [20]. Serial dilution was carried out from 10^{-1} to 10^{-6}. Actinomycetes were isolated using Luria Bertani (M1), Starch casein (SC) and Starch nitrate (SN) agar media. The media were supplemented with 25µg ml^{-1} nystatin to suppress growth of fungi and 10µg ml^{-1} nalidixic acid to suppress growth of other bacterial species [21].

Primary and secondary screening of actinomycetes for antagonism against test pathogens

Staphylococcus aureus (ATCC 25923), *Streptococcus pneumoniae* (ATCC 49617), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 49990), *Candida albicans* (ATCC 10231), and *Fusarium oxysporum* (ATCC 16608) were used as test organisms. Primary screening for antagonism was carried out using perpendicular method while secondary screening was carried out using Kirby Bauer's disc diffusion bioassay [22]. Based on broad spectrum of activity and size of the zone of inhibition, four actinomycetes abbreviated PAN 15, PAN 42, PAN 70 and PAN 100 were selected for further analyses. The selected actinomycetes were characterized using morphological characteristics, Gram's staining and Anaytical Profile Index (API) strips [23].

Extraction of crude metabolites

Liquid state fermentation

Briefly, PAN 15, PAN 42, PAN 70 and PAN 100 were each inoculated into 3L Luria Bertani (M1) broth, and incubated at 28±2°C in a shaker (200 rpm) for 7d. The broths were filtered using Millipore filters (Millipore Millex-HV Hydrophilic PVDF 0.45 Zm). To each culture filtrate, equal volume of ethyl acetate was added and centrifuged at 5000 rpm for 10 min [24]. The extracts were concentrated using a rotary evaporator (Heidolph Laborota, 4001, Büchi Vacuum Controller, V-805) at 40 ± 2^{o}C. The concentrated extracts were placed in pre-weighed evaporating dishes and the remaining solvent evaporated using a dry heat oven (Schwabach, DIN 40050-IP20, V= 240, Hz 50/60) at 40 ± 2^{o}C and the weights of the extracts determined. Each extract was suspended in ethyl acetate and the antibiotic property determined against the test pathogens using Kirby Bauer disc diffusion technique [25].

Solid state fermentation

Each of the four isolates PAN 15, PAN 42, PAN 70 and PAN 100 was plated on 3L of Luria Bertani agar (M1) using streak plate technique. The Petri plates were incubated at 28±2°C for 7d. The agar was cut into small pieces using a spatula and separately placed in conical flasks. Ethyl acetate was added at the ratio of 1:1 by volume and shaken using an orbital shaker (Gallenkamp, Model 10X 400) for 1h at 200 rpm. The solid material was filtered off using Whatman number 1 filter papers and the extracts concentrated using a rotary evaporator (Heidolph Laborota, 4001, Büchi Vacuum Controller V-805) at 40±2°C. Each extract was placed in a pre-weighed evaporating dish and the excess solvent evaporated using a hot air oven (Schwabach, DIN 40050-IP20, V=240, Hz 50/60) at 40±2°C and the weights determined. Antibiotic property of each extract was carried out using Kirby Bauer's disk diffusion bioassay [26].

Determination of cytotoxic activity of the antibiotic metabolites

Concentrations ranging from 2 to 31µg/mL of each antibiotic extract was placed in vials. Briefly, 5mL of brine and 10 previously hatched shrimps were added and incubated at 25°C. The surviving shrimps determined after 24h. The LC_{50} values of each antibiotic extracts were computed using regression and probit analysis [27].

Data analysis

The data obtained was analyzed using Statistical Package for Social Sciences (SPSS) Version 25.0 software. The diameter of the zones of inhibition were compared using One-way ANOVA. The results of cytotoxicity test were analyzed using regression and probit analysis. A P value of ≤ 0.05 was considered statistically significant.

RESULTS

Actinomycetes from Menengai crater

There was no significant difference in the number of actinomycetes isolated using starch casein, Luria Bertani and starch nitrate (P= 0.34). The mean actinomycetes isolates varied from $1.47x10^3$ CFU/g of soil in starch casein to $2.34x10^3$ CFU/g of soil in Luria Bertani.

Table 1: Number of actinomycetes (CFU/g) isolated from soils of Menengai crater using different types of media.

Dilution factor	Starch casein	Luria Bertani	Starch nitrate
10^{-1}	$3.0 x10^3$	$4.0 x10^3$	$3.3x10^3$
10^{-2}	$2.7 x10^3$	$3.5 x10^3$	$3.0x10^3$
10^{-3}	$2.5 x10^3$	$3.0 x10^3$	$2.7x10^3$
10^{-4}	$2.1 x10^2$	$3.0 x10^3$	$2.4x10^3$
10^{-5}	$1.9 x10^2$	$2.8 x10^2$	$2.1x10^2$
10^{-6}	$1.8 x10^2$	$2.6 x10^2$	$1.8 x10^2$
Mean	$1.47x10^3$	$2.34x10^3$	$1.97x10^3$

Each value represents the mean value (±SD) of five independent replicates.

Antibiotic property of broth extracts of actinomycetes from Menengai Crater

There was no significant difference in the zones of inhibition between the isolates (P=0.00071). The mean zone of inhibition varied from 18±0.3mm in PAN 100 to 19±0.3mm in PAN 70 (Table 2).

Table 2: Zones of inhibition (mm) of the test pathogens by broth extracts of the selected actinomycetes

Isolate	*S. aureus*	*S. pneu*	*P.aerugi*	*P.vulga*	*C. albicans*	*F. oxyspo*	Mean
PAN 15	20±0.2	21±0.3	18±0.3	18±0.2	19±0.3	18±0.2	19±0.2
PAN 42	19±0.2	20±0.2	19±0.2	19±0.2	18±0.2	19±0.3	19±0.1
PAN 70	21±0.3	22±0.3	20±.03	17±0.3	19±0.3	17±0.2	19±0.3
PAN 100	20±0.3	21±0.2	18±0.3	18±0.3	17±0.2	19±0.2	18±0.3

Each value represents the mean value (±SD) of five independent replicates*; S. aureus; Staphylococcus aureus, S. pneu; Streptococcus pneumoniae, P. aerugi; Pseudomonas aeruginosa; P. vulga; Proteus vulgaris* and *C. albicans; Candida albicans* and *F. oxyspo; Fusarium oxysporum.*

Antibiotic yield in liquid and solid fermentation

The yield of the crude antibiotic extract between liquid and solid-state fermentation varied significantly (P = 0.0056). The yield of the crude antibiotic extracts varied from 221.4±0.2 mg in PAN 42 to 249.5±0.1 mg in PAN 100 in liquid fermentation (Figure 1). On the other hand, the crude antibiotic extracts yield in solid fermentation ranged from 670.4±0.1mg in PAN 42 to 743.7±0.3 in PAN 100.

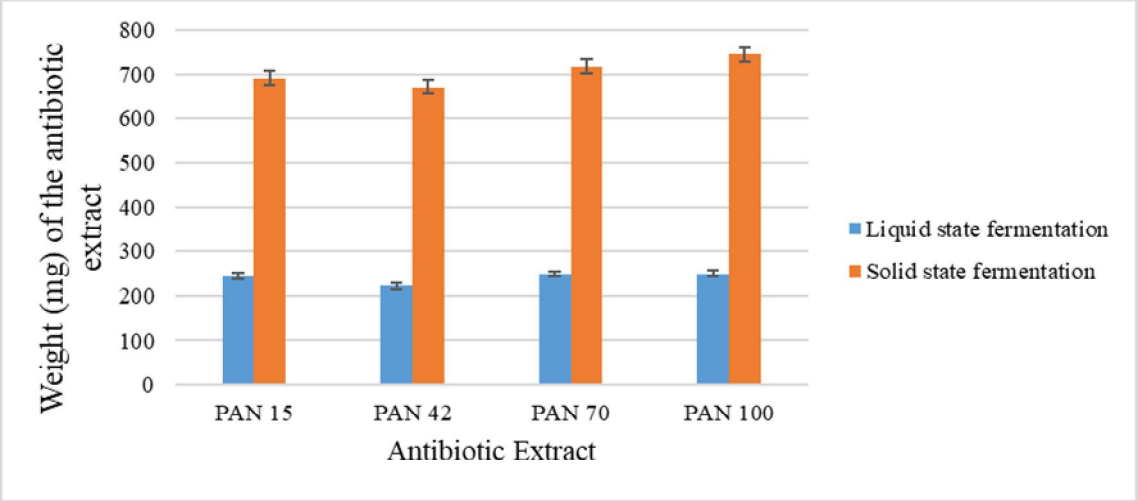

Figure 1: Weight (mg) of the antibiotic extracted from PAN 15, PAN 42, PAN 70 and PAN 100 using liquid and solid-state fermentation.

Antibiotic activity of ethyl acetate extracts in liquid state fermentation

The zones of inhibition varied significantly (P=0.0089) between the extracted antibiotic metabolites. In PAN 15, the zones of inhibition varied between 18±0.3 mm-32±0.1mm, PAN 42 (17±0.2mm-29±0.2mm), PAN 70 (16±0.1mm-34±0.1mm) and PAN 100 (16±0.3mm-20±0.2) (Table 3). However, the mean zone of inhibition ranged from 17.83±0.1mm to 26.83±0.1mm

Table 3: Zones of inhibition (mm) after exposure of the test pathogens to ethyl acetate extracts from liquid state fermentation of the selected actinomycetes.

Extract	*S. aureus*	*S. pneu*	*P. aerugi*	*P. vulga*	*C. albicans*	*F. oxyspo*	Mean
PAN 15	32±0.1	30±0.2	21±0.3	22±0.1	22±0.2	18±0.3	24.17±0.3
PAN 42	29±0.2	28±0.1	18±0.1	20±0.3	19±0.3	17±0.2	26.83±0.1
PAN 70	34±0.1	31±0.3	28±0.3	23±0.2	20±0.1	16±0.1	25.33±0.2
PAN 100	20±0.2	18±0.2	17±0.2	16±0.3	19±0.1	17±0.2	17.83±0.1

Each value represents the mean value (±SD) of five independent replicates*; S. aureus; Staphylococcus aureus, S. pneu; Streptococcus pneumoniae, P. aerugi; Pseudomonas aeruginosa; P. vulga; Proteus vulgaris* and *C. albicans; Candida albicans; F. oxyspo; Fusarium oxysporum.*

Antibiotic activity of ethyl acetate extracts in solid state fermentation

There was a significant difference in the zones of inhibition between the extracted antibiotic metabolites (P=0.0086). The zones of inhibition in PAN 15 varied from 23±0.3mm to 37±0.1mm, PAN 42 (22±0.2mm-34±0.2mm), PAN 70 (21±0.1mm-39±0.1mm) and PAN 100 (20±0.3mm-25±0.2mm) (Table 4). The mean zone of inhibition ranged from 22.50±0.1mm to 30.33±0.2mm.

Table 4: Zones of inhibition (mm) after exposure of the test pathogens to ethyl acetate extracts from solid state fermentation of the selected actinomycetes.

Extract	*S. aureus*	*S. pneu*	*P. aerugi*	*P. vulga*	*C. albicans*	*F. oxyspo*	Mean
PAN 15	37±0.1	35±0.2	26±0.3	27±0.1	27±0.2	23±0.3	29.17±0.3
PAN 42	34±0.2	33±0.1	23±0.1	25±0.3	24±0.3	22±0.2	26.83±0.1
PAN 70	39±0.1	36±0.3	33±0.3	28±0.2	25±0.1	21±0.1	30.33±0.2
PAN 100	25±0.2	23±0.2	22±0.2	20±0.3	24±0.1	21±0.2	22.50±0.1

Each value represents the mean value (±SD) of five independent replicates*; S. au; Staphylococcus aureus, S. p; Streptococcus pneumoniae, P. ae; Pseudomonas aeruginosa; P. vu; Proteus vulgaris* and *C. al; Candida albicans; F. ox; Fusarium oxysporum.*

Cytotoxicity of the antibiotics from the selected actinomycetes

The mortality of brine shrimps varied in PAN 15 varied from (50 - 100%), PAN 42 (60 - 100%), PAN 70 (26 - 100%) and PAN 100 (16 - 99%) (Table 5). The LC_{50} values ranged from 2. 68±0.27 µg/ml in PAN 15 to 4.97±0.11µg/ml in PAN 100.

Table 5: LC_{50} values of crude antibiotic metabolites on brine shrimp lethality bioassay.

Antibiotic extract	Sample conc. (µg/ml)	Log C	% Mortality	LC_{50} (µg/ml)
	2	0.301029996	50	
PAN 15	4	0.602059991	59	2. 68±0.27
	8	0.903089987	64	
	16	1.204119983	100	
	31	1.491361694	100	
	2	0.301029996	60	
PAN 42	4	0.602059991	67	3.59±0.29
	8	0.903089987	100	
	16	1.204119983	100	
	31	1.491361694	100	
	2	0.301029996	26	
PAN 70	4	0.602059991	33	3.69±0.15
	8	0.903089987	84	
	16	1.204119983	92	
	31	1.491361694	100	
	2	0.301029996	16	
PAN 100	4	0.602059991	33	4.97±0.11
	8	0.903089987	50	
	16	1.204119983	64	
	31	1.491361694	99	

Each value represents the mean value (±SD) of five independent replicates, Conc.; concentration.

DISCUSSION

This study indicated that Luria Bertani recovered many actinomycetes from the composite soil sample than starch casein and starch nitrate agar medium. This may be attributed to differences in the ingredients used in making the isolation medium [28]. In addition, Luria Bertani could have provided nutrients that were similar to those the actinomyctes were getting in the sampling site [29]. However, [30] suggested that high numbers of actinomycetes are to be recovered in environmental samples, nutrients almost similar to those found in the natural environment should be provided. The broth extracts of the selected actinomycetes inhibited growth of the test pathogens presenting almost equal sizes of zones of inhibition. This contradicted the results of a previous study [31]. This may be attributed to differences in the actinomycetes isolates [32]. Generally, broth extracts of antibiotic metabolites produce smaller zones of inhibition than when solvents are used to extract the antibiotics [33]. This can be attributed to the particles of the broth media retaining some of the active components of the antibiotics after filtration [34]. In the current study, solid agar medium produced high weights of the antibiotic crude extracts than liquid agar medium. The results concurred with a previous study carried out on characterization of *Streptomyces sp.* KB1 and its cultural optimization for bioactive compounds production [35]. Rakesh *et al.* [36] maintained that agar has some compounds that provides optimal growth of the actinomycetes leading in increased production of antibiotics. According to Sharma *et al.* [37] liquid media has high water activity that leads to increased contamination. The increased contamination leads to reduced growth of actinomycetes which reduces production of antibiotics [38]. The shacking conditions provided during liquid state fermentation also breaks the actinomycetes filaments reading to reduced growth and production of acinomycetes [39]. Gram positive bacteria presented bigger zones of inhibition than the Gram-negative pathogens. Likewise, the *C. albicans* gave bigger zones of inhibition than *F. oxysporum*. These results were in agreement with those of a previous study by Tortorella *et al.* [40]. Siro *et al.* [41] argued that the differences in inhibition of Gram positive and negative bacteria can be attributed to variations in cellular components of the two categories of bacteria. The Gram-negative bacteria have a thinner peptidoglycan layer than Gram positive bacteria which makes them less prone to antibiotics [42]. In addition, they have an extra outer membrane layer which accords them resistance to antibiotics [43]. The possession of filaments in addition to production of resistant spores accords moulds the ability to resist antibiotics when compared to cellular fungi such as *C. albicans*

[44]. The zones of inhibition observed from the ethyl acetate antibiotic extracts from the selected actinomycetes using solid medium were bigger than in liquid medium. These findings support those of another study carried out by Lema *et al.* [45]. Mangamuri *et al.* [46] asserted that among the factors important and most crucial for microbial growth and activity in a solid-state culture are substrate type, particle size and moisture level. Broadly speaking, smaller substrate particles provide a larger surface area for attachment of actinomycetes [47]. On the other hand, too small substrate such as in liquid medium affects actinomycetes respiration negatively leading to reduced growth [48]. Larger particles such as in solid medium supports increased respiration resulting from increased aeration because of increased inter-particle space which supports production of antibiotics [49]. The calculated LC_{50} values of the actinomycete metabolites indicate that antibiotics produced by isolate PAN 25 were the most toxic while those produced by PAN 100 were the least toxic to *Artemia salina*. In addition, the LC_{50} (µg/ml) values were less than 20 indicating that the antibiotic extracts were good candidates for testing anti-tumor properties [50]. The finding differed with a previous study carried out by Paudel *et al.* [51]. The difference may be attributed to variations in the types of antibiotic extracts produced by the actinomycetes isolates [52].

CONCLUSION

Soils from Menengai crater had actinomycetes that produced antibiotics. The selected actinomycetes produced high amounts of antibiotics in solid than in liquid state fermentation.

RECOMMENDATIONS

There is need to characterize the selected actinomycetes using molecular techniques. The antibiotics from the actinomycetes need to be analyzed using LC-MS and NMR.

REFERENCES

1. Assou, S.A., Anissi, J., Sendide, K. & Hassouni, M.E. (2023). Diversity and antimicrobial activities of *Actinobacteria* isolated from mining soils in Midelt region, Morocco. Sci. World J., 23: 1-19.
2. Medjemadj, M., Escuder-Rodríguez, J., Boudemagh, A. & González-Siso, M. (2020). Actinobacteria isolated from Algerian hot spring waters: A potential source of important enzymes, Eco. Env. Cons., 26 (3):1145-1157.
3. Benhadj, M., Gacemi-Kirane, D., Menasria, T., Guebla, K. & Ahmane, Z. (2019). Screening of rare actinomycetes isolated from natural wetland ecosystem (Fetzara Lake, northeastern Algeria) for hydrolytic enzymes and antimicrobial activities. J. King Saud Univ. Sci., 31:706-712.
4. Azimi, S., Basei Salehi, M. & Bahador, N. (2017). Isolation and Identification of *Streptomyces ramulosus* from soil and determination of antimicrobial property of its pigment. Mod. Med. Lab. J., 1:36-41.
5. Charousová, I., Medo, J., Halenárová, E. & Javoreková, S. (2017). Antimicrobial and enzymatic activity of actinomycetes isolated from soils of coastal islands. J. Adv. Pharm. Technol. Res., 8:46-51.
6. Jagannathan, S., Manemann, E., Rowe, S., Callender, M. & Soto, W. (2021). Marine actinomycetes, new sources of biotechnological products. Mar. Drugs., 19:365.
7. Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C. & Meier-Kolthoff, J.P. (2016). Taxonomy, physiology, and natural products of actinobacteria. Microbiol Mol. Rev., 25:1-43.
8. Waithaka, P.N., Mwaura, F.B., Wagacha, J.M., Gathuru, E.M. & Githaiga, B.M. (2017). Antimicrobial properties of actinomycetes isolated from Menengai crater in Kenya. CellBio., 6:13- 26.
9. Choudhary, A., Naughton, L.M., Montánchez, I., Dobson, A.D.W. & Rai, D.K. (2017). Current status and future prospects of Marine Natural Products (MNPs) as antimicrobials. Mar. Drugs., 15:272.
10. Liu, L., Salam, N., Jiao, J.Y., Jiang, H.C., Zhou, E.M., Yin, Y.R., Ming, H. & Li, W.J. (2016). Diversity of culturable thermophilic actinobacteria in hot springs in Tengchong, China and Studies of their Biosynthetic Gene Profiles. Microb. Ecol., 72:150–162.
11. Harir, M., Miloud, B., Zohra, F., García-Arenzana, J.M., Veloso, A. & Rodríguez-Couto, S. (2017). Isolation and characterization of actinobacteria from Algerian Sahara Soils with antimicrobial activities. Int. J. Mol. Cell Med., 6:109–120.
12. Hasani, A., Kariminik, A. & Issazadeh, K. (2014). *Streptomycetes*: characteristics and their antimicrobial activities. Int. J. Adv. Biol. Biomed. Res., 2(1):63-75.
13. Chaudhary, N. & Prabhu, S. (2016). Thermophilic Actinomycetes from Hot Water Spring Capable of producing enzymes of industrial importance. Int. J. Res. Stud. Biosci., 4:29-35.
14. Ding, T., Yang, L.J., Zhang, W.D. & Shen, Y.H. (2019). The secondary metabolites of rare actinomycetes: chemistry and bioactivity. RSC Adv., 9:21964-21988.
15. Mohammadipanah, F. & Momenilandi, M. (2018). Potential of rare actinomycetes in the production of metabolites against multiple oxidant agents. Pharm. Biol., 56(1):51–59.
16. Ram, V., Kumari, K.S & Kurup, R. (2022). Isolation and Screening of *Actinomycetes* from marine soil sediments and sponges for antimicrobial activities. West Indian Med. J., 69(8):571-577.

17. Bawazir, A.M.A., Shivanna, G.B. & Shantaram, M. (2018). Impact of different media for growth and production of different soluble pigments in actinomycetes isolated from soils of Hadhramout, Yemen. Eur. J. Biomed. Pharm. Sci., 5(7):615-619.
18. Waithaka, P.N., Mwaura, F.B., Wagacha, J.M., Gathuru, E.M., Ngumbu, F.M. & Mwangi, A.K. (2017). Chemical characteristcs and microbial diversity of soils from Menengai crater in Kenya. Chemical characteristics and microbial diversity of soils from Menengai crater in Kenya. Borneo Sci., 38(1):1-7.
19. Wekesa, C., Okun D., Juma, K., Shitabule, D., Okoth, P., Nyongesa, P., Katoo, A., Mulama, S., Wamalwa, E., Mahalo, C., Koyo, M., Rotich, A., Kawaka, F. & Muoma, J. (2016). Abundance and symbiotic potential of common bean (*Phaseolus vulgaris*) nodule associated bacteria in Western Kenya soil. MAYFEB J. Agric. Sci., 1:1-9.
20. Mahato, S., Lamichhane, G.C. & Thakur, A. (2021). Isolation and Screening of Antibiotic Producing Actinomycetes from Soils of Hills and Plains of Eastern Nepal. J. Clin. Pharm., 5(1): 1019-1026.
21. Waithaka, P.N. (2022). Effect of cultural conditions and physical factors on production of antibiotic metabolites by selected actinomycetes. Arch Ecotoxicol,. 4(2) 67-76.
22. Pipite, A., Lockhart, P.J., McLenachan, P.A., Christi, K., Kumar, D., Prasad, S. & Subramani, R. (2022). Isolation, antibacterial screening, and identification of bioactive cave dwelling bacteria in Fiji. Front. Microbiol., 13:1012867.
23. El Karkouri, A., Assou, S.A. & El Hassouni, M. (2019). Isolation and screening of actinomycetes producing antimicrobial substances from an extreme Moroccan biotope. Pan Afr. Med. J., 33: 329-335.
24. Ouchari, L., Boukeskasse, A., Bouizgarne, B. & Ouhdouch, Y. (2019). Antimicrobial potential of actinomycetes isolated from the unexplored hot Merzouga desert and their taxonomic diversity. Biol. Open., 8(2):23-30.
25. Kaitouni, L.B., Anissi, J., Sendide, K. & El Hassouni, M. (2020). Diversity of hydrolase-producing halophilic bacteria and evaluation of their enzymatic activities in submerged cultures. Ann. Microbiol., 70(1):33-44.
26. Rammali, S., Hilali, L. & Dari, K. (2022). Antimicrobial and antioxidant activities of *Streptomyces species* from soils of three di1erent cold sites in the Fez-Meknes region Morocco.Sci. Rep., 12(1):20-22.
27. Syiemiong, D. & Jha, D. K. (2019). Antibacterial potential of actinobacteria from a limestone mining site in Meghalaya, India. J. Pure Appl. Microbiol., 13(2):789–802.
28. Arasu, M.V., Rejiniemon, T.S. & Al-Dhabi, N.A., Duraipandiyan, V., Ignacimuthu, S., Agastian, P., Kim, S., Huxley, V.A.J., Lee, K.D. & Choi, K.C. (2014). Nutritional requirements for the production of antimicrobial metabolites from *Streptomyces*. Afr J. Microbiol. Res., 8:750-758.
29. PrashithKekuda, K.R. & Onkarappa, R. (2017). Effect of nutritional and cultural conditions on bioactive metabolite production by *Streptomyces variabilis* strain PO-178. Indian J. Nov. Drug Deliv., 9:107-114
30. Das, P., Kundu, S., Maiti, P.K., Mandal, S., Sahoo, P. & Mandal, S. (2022). An antibacterial compound pyrimidomycin produced by *Streptomyces* sp. PSAA01 isolated from soil of Eastern Himalayan foothill. Sci. Rep., 12(1):23-34.
31. Shi, H., Hu, Y., Odermatt, P.D., Gonzalez, C.G., Zhang, L., Elias, J.E., Chang, F. & Huang, K.C. (2021). Precise regulation of the relative rates of surface area and volume synthesis in bacterial cells growing in dynamic environments. Nat. Commun., 12:1975
32. Waithaka, P.N., Mwaura, F.B., Wagacha, J.M. & Gathuru, E.M. (2017). Isolation of Actinomycetes from the soils of Menengai Crater in Kenya. Greener J. Microbiol. Antimicrob., 3 (2):008-017.
33. Benhadj, M., Metrouh, R., Menasria, T., Gacemi-Kirane, D.G., Slim, F.Z. & Ranque, S. (2020). Broad-spectrum antimicrobial activity of wetland-derived *Streptomyces Sp.* ACTIF450. Ex. J., 19:360-371.
34. Roy, S. & Banerjee, D. (2015). Bioactive endophytic actinomycetes of *Cinnamomum* sp.; Isolation, identification, activity guided purification and process optimization of active metabolite. Am. J. Microbiol., 6(1):4-13.
35. Lertcanawanichakul, M. & Sahabuddeen, T. (2023). Characterization of *Streptomyces sp.* KB1 and its cultural optimization for bioactive compounds production. PeerJ., 10.7717.
36. Rakesh, K.N., Dileep, N., Junaid, S. & Prashith, K.T.R. (2014). Optimization of culture con-ditions for production of bioactive metabolite by bioactive *Streptomyces* species SRDP-TK-07. Int J. Adv. Pharm. Sci., 5(1):1809-1816.
37. Sharma, P., Ranghar, S. & Baunthiyal, M. (2020). Identification and optimization of fermentation medium for production of antibacterial compounds from endophytic *Streptomyces* sp. GBTPR-167. Int. J. Curr. Microbiol. Appl. Sci., 9(06):2594-2608.
38. Ababutain, I.M., Abdul-Aziz, Z.K. & Al-Meshhen, N.A. (2013). Optimization of environmental and nutritional conditions to improve growth and antibiotic productions by *Streptomyces* sp. Int. Res. J. Microbiol., 4:179-87.
39. Ganesan, G., Velayudhan, S.S. & David, J.S.R. (2017). Statistical optimization of medium constituents and conditions for improved antimicrobial compound production by marine *Streptomyces* sp. JGR-04. Arch. Biol Sci., 69 (4):723-731.
40. Tortorella, E., Tedesco, P., Esposito, F.P., January, G.G., Fani, R., Jaspars, M. & de Pascale, D. (2018). Antibiotics from Deep-Sea Microorganisms: Current Discoveries and Perspectives. Mar. Drugs.,16:355.
41. Siro, G., Pipite, A., Christi, K., Srinivasan, S. & Subramani, R. (2022). Marine actinomycetes associated with stony corals: A potential hotspot for specialized metabolites. Microorganisms., 10: 1349.
42. Genilloud, O. (2017). Actinomycetes: Still a source of novel antibiotics. Nat. Prod. Rep., 34:1203-1232.
43. Bundale, S., Begde, D., Nashikkar, N. & Kadam, T. 2015. Optimization of culture conditions for production of bioactive metabolites by Streptomyces spp. isolated from soil. Adv. Microbiol., 5:441-451.
44. Subramanian, D., Kim, M.S., Kim, D.H. & Heo, M.S. 2017. Isolation, characterization, antioxidant, antimicrobial and cytotoxic effect of marine actinomycete, *Streptomyces carpaticus* MK-01, against fish pathogens. Braz. Arch. Biol. Technol., 60: e17160539.

45. Lema, W.S., Mahadhy, A., Damas, M. & Joan J. E. (2022). Characterization and antimicrobial potential of actinobacteria isolated from Momela Soda Lakes, Tanzania. Tanz. J. Sci., 48(3): 607-622.
46. Mangamuri, U.K., Muvva, V., Poda, S. & Agasar, D. (2014). Optimization of process parameters for improved production of bioactive metabolites by *Streptomyces tritolerans* DAS 165T. British Microbiol. Res. J., 4:428-442.
47. Lee, Y., Lee, M., Choi, Y., Chun, G. & Jeong, Y. (2014). Optimization of cultivation medium and fermentation parameters for lincomycin production by *Streptomyces lincolnensis*. Biotechnol. Bioprocess Eng., 19:1014-1021.
48. Singh, C., Singh, P.R., Jadon, P. & Kumar, A. (2017). Optimization of cultural conditions for production of antifungal bioactive metabolites by *Streptomyces spp.* isolated from soil. Int. J. Curr. Microbiol. Appl. Sci., 6:386-396.
49. Karanam, G., Arumugam, M.K. & Natesh, N.S. (2020). Anticancer effect of marine sponge-associated *Bacillus pumilus* AMK1 derived dipeptide Cyclo (-Pro-Tyr) in human liver cancer cell line through apoptosis and G2/M phase arrest. Int. J. Pept. Res. Ther., 26(1):445–457.
50. Chawawisit, K., Bhoopong, P., Phupong, W. & Lertcanawanichakul, M. (2015). Antimicrobial and cytotoxic activities of bioactive compounds produced by Streptomyces sp. KB1. Int. J. Pharm. Pharm. Sci., 7(11):118-122.
51. Paudel, B., Maharjan, R., Rajbhandari, P., Aryal, N., Aziz, S., Bhattarai, K., Baral, B., Malla, R. & Bhattarai, H.D. (2021). Maculosin, a non-toxic antioxidant compound isolated from *Streptomyces* sp. KTM18. Pharm. Biol., 59(1):931-934.
52. Chen, S., Zhang, D., Chen, M., Zhang, S. & Lian, X.Y. (2020). A rare diketopiperazine glycoside from marine-sourced Streptomyces sp. ZZ446. Nat Prod Res., 34(7):1046–1050.