Characterization and Antibacterial Activity of Soil Actinomycetes from Diverse Land Use Systems in Meru South, Eastern Kenya

ABSTRACT

Aim: The increasing emergence and global spread of antibiotic-resistance by microorganisms pose a severe public health threat. Continuous exploration of different environments is required to find new sources of antibacterial compounds. Therefore, the study isolated, characterized and identified potential Actinomycetes candidates with antibacterial activity against bacterial pathogens from diverse land use systems.

Study design: Soil samples from land use systems were collected using a cross-sectional survey technique using line transect sampling in order to isolate Actinomycetes. A 30 × 7× 3 factorial experiment with a completely randomized design was used for evaluating Actinomycetes isolates with antibacterial activity.

Methodology: The in-vitro cultivation of Actinomycetes was evaluated using four selective media. The Actinomycetes isolates were characterized using morphological, biochemical, and molecular markers. The antibacterial activity screening of crude extracts was conducted against six bacterial pathogens using the agar well diffusion method. The antibacterial activity of Actinomycetes isolates were analyzed using Analysis of Variance. The molecular identification was performed using 16S rRNA sequence homology.

Results: The morphological analysis showed variations in colony morphology, including differences in color, size, and texture. Biochemical tests provided insights into the metabolic capabilities of the isolates, indicating variations in enzymatic activities and substrate utilization. Antagonistic activity of Actinomycetes extracts exhibited significant differences (P =.05) against test bacterial pathogens. Notably, isolate C52 from degraded forest zone showed the highest antibacterial activity against *Staphylococcus aureus* (12.21 mm), isolate L6 (16.23 mm) against *Listeria monocytogenes* and isolate C50 (15.5 mm) against *Raoutella planticola*. *Streptomyces celluloflavus*, *S. griseobrunneus, S. pratensis, S. crystallinus*, and *S. eurocidicus* were identified in the study.

Conclusion: The crude extracts obtained from Actinomycetes showed significant antibacterial activity against the selected test organisms. This suggests the presence of bioactive compounds with potential antibacterial properties within these extracts.

Keywords: Soil Actinomycetes, Characterization, Antibacterial Activity, Land use systems

1. INTRODUCTION

Antibiotic resistance has emerged as a major public health issue, causing serious health problems and a concerning increase in morbidity and mortality rates [1] [2]. The misuse and overuse of antibiotics have accelerated the development

UNDER REVIEW

of antibiotic resistance, making it one of the most critical challenges faced by healthcare facilities in the twenty-first century [3].The prevention and treatment of infectious illnesses caused by antibiotic-resistant bacteria has become a top priority [4]. Investigating novel bioactive compounds, particularly those with different mechanisms of action, is critical for addressing present medical challenges and preventing future pandemics. Antibiotics can be found in Actinomycetes, a substance that is naturally produced by microorganisms [5] [6]. In order to meet the demand for new antibiotics, it is importantl to investigate a wide range of natural environments and land use systems. This is important because certain Actinomycetes populations may exist in specific environments and have the ability to produce antibiotics. These environments, as examples, include urban areas, agricultural fields, forest soils, and plant ecosystems all of which have proven significant in sourcing Actinomycetes for screening against resistant bacterial pathogens [7] [8] [9]. Land use systems, which include a wide range of anthropogenic activities and management methods, have a considerable impact on microbial populations and their functional potential [10, 11]. Actinomycetes, regarded as vital constituents of the soil microbiota, can vary in abundance, diversity, and functional characteristics across diverse land use systems [12] [13].

Actinomycetes can be identified using morphological, biochemical, and molecular markers, which can give valuable information about their variety, physiology, and metabolic capacity [14] [15]. Morphological examination, which is largely based on microscopy and allows for the observation of cellular and colony features, spore formation, and branching patterns, provides preliminary taxonomic insights [16]. Taxonomy and phylogenetic studies of Actinomycetes have been revolutionized by molecular markers such as 16S rRNA gene sequences [17]. By focusing on conserved portions of the 16S rRNA gene, molecular markers enable precise genus- and species-level identification of Actinomycetes [18].Therefore, comprehensive studies on characterizing Actinomycetes isolated from different land use systems was essential to gain a deeper understanding of their biotechnological potential.

Soil Actinomycetes, which are capable of producing a broad spectrum of secondary metabolites, are accountable for approximately 70% of the naturally derived antibiotics currently in use in healthcare applications [18]. Actinomycetes, specifically *Streptomyces* and *Micromonospora,* are known to be the source of over 80% of antibiotics used globally [20]. A significant portion of its bioactive components, such as avermectin, streptomycin, tetracycline, and chloramphenicol, have been shown to have antibacterial, antifungal, antiviral, and antiparasitic activities [21]. To address the resistance threat, innovative antibiotics that are effective against resistant pathogenic bacteria must be discovered. The current study characterized and screened Actinomycetes from different land use systems in order to identify new Actinomycetes strains that produce active antibiotics that have not yet been discovered.

2. MATERIAL AND METHODS

2.1 Study Site

The study was conducted out in Meru South Sub-County, which is within Tharaka Nithi County, Eastern Region of Kenya. The locations for soil sampling sites in the current study are depicted in figure 1 below. Meru South Sub-County is defined by low and unpredictable rainfall as well as a high evapotranspiration rate [22]. The study area has bimodal temperatures which vary from 24°C to 26°C, with rainfall ranging from 1200 to 1400 mm. The predominant soil texture is sand clay loam, and the most prominent soil type is humic nitisols, which are deep, weathered soils with moderate to high fertility [22].

UNDER PEER REVIEW

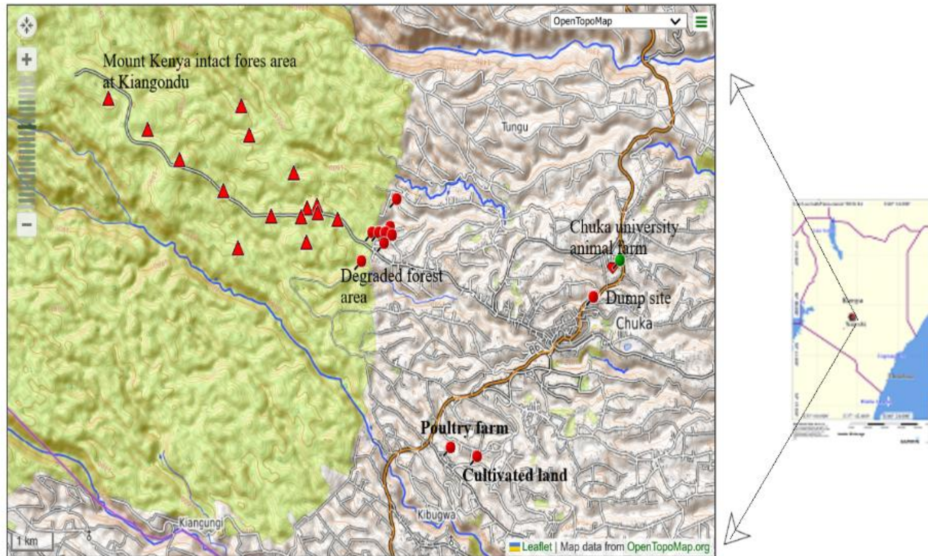


Figure 1: The soil sampling sites in the study area within Meru-South sub-county.

2.2 Study design

Soil samples from land use systems were collected using a cross-sectional survey technique using line transect sampling in order to isolate Actinomycetes. A 30 × 7× 3 factorial experiment with a completely randomized design was used for evaluating Actinomycetes isolates with antibacterial activity.

2.3 Soil samples collection

Soil samples were collected from uncultivated and degraded soils in the Mt. Kenya forest (S0° 18.247' E37° 34.111'), cultivated soils from agricultural land (S0° 19.347' E37° 39.483'), cattle manure from Chuka University, chicken manure from Ikuu Farm, and soils from a municipal solid waste dumpsite in Chuka town (S0° 19.699' E37° 39.176').The sampling locations were marked using the Etrex 30x Garmin Global Positioning System to ensure accurate spatial referencing (Table 1). The soil samples were collected using a spiral soil auger, and after each sample collection it was cleaned with 10% formalin to eliminate cross-contamination. Soil samples were collected at a depth of five centimeters from cultivated soil, dumpsites, and forest zones during the sampling process. The collected soil samples were packed in sterile containers with zip-top lids and then they were transported to the Botany laboratory at Chuka University for further analysis.

Table 1: Soil sampling from different land use systems of Meru South Sub-county

UNDER PEER REVIEW

Site(s[1])	Soil sampling area	Ecological importance	Sd[2] (cm)	Elevation	Latitude	Longitude
A	Chuka forest zone	Intact soils	5	1593m	0º19. 30'S	37º 36'0''E
B	Ikuu farm	Cultivated soils	5	1321m	0º11.0''S	37º 19'00 E
C	Chuka forest zone	Destructed area	5	1558m	0º19.30'S	37º36'00''E
D	Chuka town	Dump site soils	5	1356m	0º 19.699'S	37º 39.17' E
E	Chuka University	cattle manure	5	1406m	0º 19.347'S	37º39.483'E
F	Ikuu farm	Chicken manure	5	1321m	0º11.0'S	37º 19.00'E

[1]Sampling sites, [2] Soil depth

2.4 Pre-treatment of soil samples

The soil samples collected from various land use systems were allowed to air dry for 7 days at room temperature [23] [24]. This was done to reduce the number of bacteria and fungi that are dependent on moisture. The soil samples were then ground up into a fine powder after being air-dried. Ten (10) g of fine soil sample was mixed with 0.1 g of CaCO3, and the resulting mixture was heated for one hour at 55^0 C in a hot air oven (Model Memmert UNB400).

2.5 Isolation of *Actinomycetes* from soil samples

The soil samples for Actinomycetes isolation were prepared using the standard serial dilution method [25]. One gram of soil samples was mixed with distilled sterile water up to 10^{-3} and shaken for 5 minutes with a vortex. Following serial dilution, 0.1 ml of each sample was plated separately in Modiied Luria Bertani Agar (M1) (Starch 10 g, Peptone 2.0 g, yeast Extract 4.0 g, Agar 18.0 g, distilled water 1000 ml, and pH; 7.0), Starch Casein A gar (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, distilled water 1000 ml, and pH; 7.0) and International Streptomyces Project (ISP-1) (Yeast Extract, 3.0g, Tryptone, 5.0g distilled water 1000 ml, and pH; 7.0) and (ISP-4) media (Soluble Starch, 10.0g, K_2HPO_4, 1.0g, $MgSO_4 \cdot 7H_2O$, 1.0g, NaCl, 1.0g, $(NH_4)_2$ SO_4, 2.0g, CaCO3, 2.0g, distilled water 1000 ml, and pH; 7.0) (HiMedia Laboratories). The plates were incubated at 28°C and colony growth was recorded on day 7 following inoculation. Actinomycetes isolates were differentiated from other microbial colonies during incubation using morphological characteristics [26]. Isolates were sub-cultured to obtain pure cultures on nutrient agar. Pure cultures were stored at 4°C in slant culture on Starch Casein Agar and in glycerol broth [27].

2.6 Morphological Characterization of Actinomycetes Isolates

In order to investigate microscopic characteristics, pure cultures from isolated strains obtained from starch casein agar, International Streptomyces project-1 (ISP-1), International Streptomyces project-4 (ISP-4) and Modified Luria Bertani agar medium were used. Colony colour, size, margin, elevation, pigment production, and the presence or absence of aerial and substrate mycelium were among the morphological characteristics examined. A compound microscope was used to examine pure cultures in order to identify the morphology of the hyphae and spores. The Gram stain method was used to investigate the organization, size, and shape of the spore chain [28].

2.7 Biochemical Characterization of Actinomycetes Isolates

UNDER PEER REVIEW

Biochemical characteristics were identified by determining the capacity of the isolates to utilize carbon sources on D-Glucose, D-Fructose, Sucrose, Maltose, Dextrose, and Lactose (HiMedia laboratory) using nutrient agar as a basal medium [29]. Casein hydrolysis [30], starch hydrolysis [31], urea hydrolysis [32], catalase, citrate utilization, lipase [33], hydrogen sulphide production [34], Indole motility [35], Methyl red and Voges- Proskauer [36] were also determined.

2.8 Screening of selected Actinomycetes for antagonistic activity against test pathogens

2.8.1 Primary Screening of Antibacterial of Actinomycetes Isolates.

Primary screening of Actinomycetes was performed on the Mueller–Hinton agar medium employing the perpendicular streak method [24]. In the sterile agar medium, the pure isolate of Actinomycetes was streaked along the diameter of the plate. The plate was incubated at 28°C for 5 days. Pure colony of test bacteria *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (NCTC 11994), *Vibrio furnissii* (NCTC 11218), *Rauotella planticola* (NCTC 19528), *Staphylococcus aureus* (ATCC 25923), and *Streptococcus mitis* (NCTC 12261) was transferred into fresh nutrient broth and incubated at 37°C for 24 hours until the visible turbidity. After adjusting the turbidity equal to that of 0.5 McFarland with the cell count of 1.5×10^8, the test organisms were streaked perpendicular to the isolate [37]. The plates were further incubated at 37°C for 24 hours, and the antibacterial activity was estimated from the zone of inhibition of test organism [38]. Precautionary gear was used while handling the test pathogens to ensure safety in the laboratory setting. Surfaces and laboratory equipment were also frequently disinfected, the frequency of contact was reduced, and laminar flow hoods were used.

2.8.2 Secondary Screening of Actinomycetes Isolates for Antibacterial Activity

Thirty isolates with inhibition zones greater than 6.5mm in primary screening were selected and fermented using the submerged fermentation technique in boiling tubes containing 30 ml of sterile medium starch casein broth [39]. The inoculated boiling tubes were cultured for 7 days at 28°C and 150 rpm on a rotary incubator shaker (Biotechnich, India). The fermented broth was centrifuged at 10,000 rpm at 4 °C (Remi, RM12C, India). The supernatant was tested for antibacterial activity using the same test microorganisms as in the primary screening [40]. With the use of sterile cork borers, five wells of 6 mm diameter were dug on the agar plate. The test organism was swabbed on the agar surface and 100 µL of supernatant was poured in the wells. The plates containing bacterial strains were incubated at 37ºC for 24 hours. Twenty-five (25) µl of Streptomycin (concentration of 10 µg) was used as positive control left at room temperature for 30 min to allow the compounds to diffuse through the agar. After incubation, the zone of inhibition was measured and expressed as millimetres in diameter [41].

2.9 Molecular characterization of Actinomycetes Isolates

The isolates were inoculated into starch casein agar media (Himedia Laboratories) and incubated at 28 °C for 7 days. Genomic deoxyribonucleic acid (gDNA) was extracted using standard phenol-chloroform procedures [42]. The 16S rRNA gene primers were then used for Polymerase Chain Reaction (PCR) amplification. The conditions for the polymerase chain reaction were an initial denaturation at 94°C for 5 min, 30 cycles at 95°C for 30 seconds, 55°C for 30 secs, and 72°C for 120 secs, and a final extension at 72°C for 7 min. The PCR amplified products underwent electrophoresis on a 0.7% agarose gel for analysis. The purified PCR products were sequenced using the 27F and 1429R primers and Sanger sequencing techniques at Genotech in Macrogen, Germany [43]. The universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1429R (5'-GGTTACCTTGTTACGACTT-3') were used in the study to determine the bacterial 16S rRNA sequence [44]. The consensus 16s rRNA gene sequence from each putative isolate was saved in

UNDER PEER REVIEW

FASTA file format and BLASTed in the NCBI database to determine isolate identity before being submitted to the same database for accession numbers. The 16s rRNA gene sequence records were immediately entered and submitted electronically using the Sequin tool/program from the National Center for Biotechnology Information (NCBI) [45]. The accession numbers of each isolate's 16s rRNA gene sequence were obtained from the GenBank database. Molecular evolutionary genetics analysis (MEGA) X was used to generate evolutionary character matrices and distance matrices from the aligned sequences. The Jukes Cantrol model was chosen to eliminate gaps and ambiguous positions. The neighbour joining original tree was constructed, and the reliability of the resulting neighbour joining tree topologies was reviewed and evaluated using bootstrap analysis on 1000 re-samplings of the neighbour-joining [46] [47]. The original tree and the bootstrap tree were compared to determine the tree's reliability. MEGA X was used for phylogenetic analysis to evaluate the evolutionary relationship of the Actinomycete sample to other bacteria.

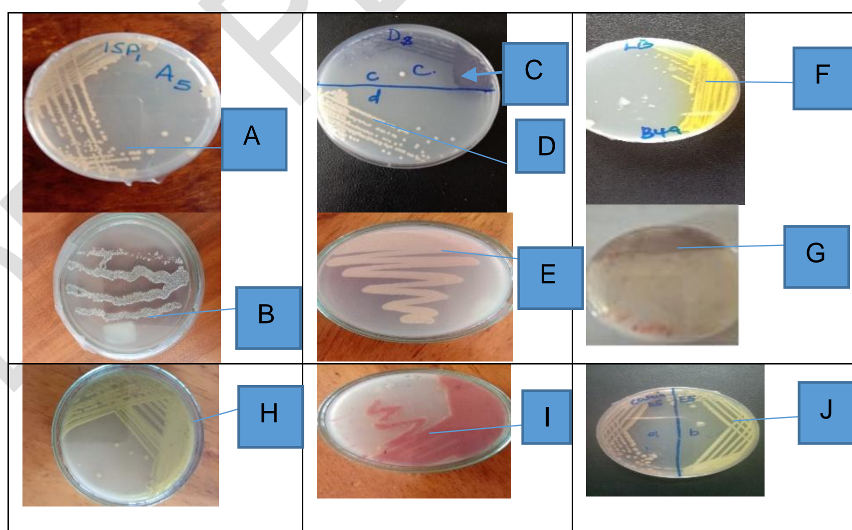
2.10 Statistical analysis

The data obtained on the diameters of zones of inhibition was subjected to analysis of variance (ANOVA) at significance value (P =.05) using SAS version 9.4 [49] to estimate the antibacterial activity of Actinomycetes isolates against test pathogens. Significant means were separated using the Least Significant Difference (LSD) at α = 0.05.

3. RESULTS

3.1 Morphological Characterization

Actinomycetes colonies had a variety of morphological characteristics (Table 2). The colonies were round, with a round edge and a raised or flat elevation. The colonies were grey, purple, pink, brown, cream, yellow, red, green-yellow, and cream-white in different isolates (Plate 1). The reverse side of the colonies also exhibited colours such as brown, yellow, pink, or grey.



Note:A=orange (ISP-1), B= Grey (SCA) C=Purple (SCA), D=cream-white (SCA), E=light pink (M1), F= yellow (M1), G= brown (ISP-1), H= light-green (ISP-4), I= red (ISP-4) and J= green- yellow (SCA). Media used LB=Luria Bertani; SCA= Starch casein agar, ISP-1 and ISP-4 International Streptomyces project media 1 starch and 4 respectively.

Plate 1: Morphological characterization of Actinomycetes isolates from soil samples collected from different land use systems

UNDER PEER REVIEW

Table 2: Morphological characteristics of selected Actinomycetes isolates

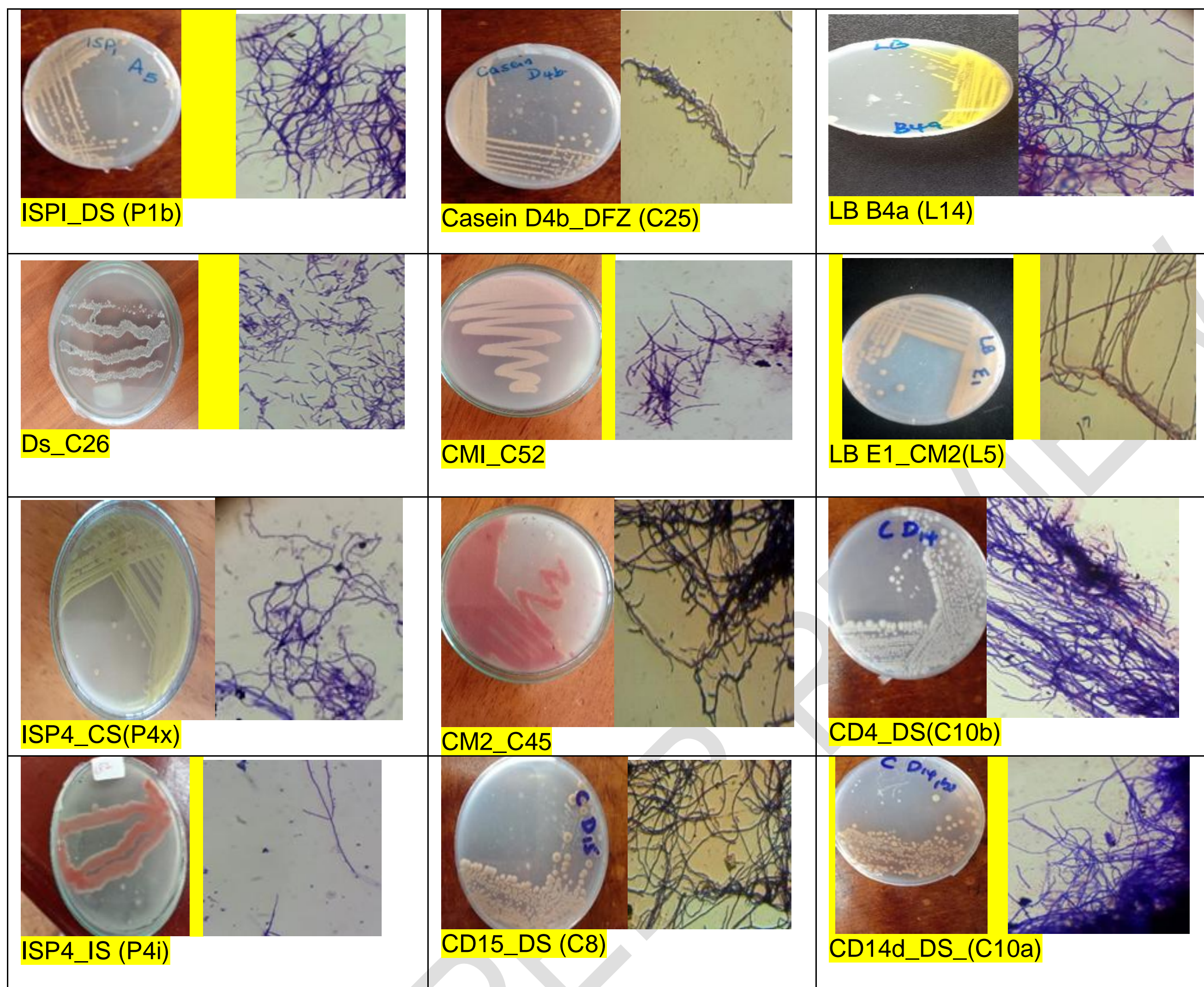
Isolates	Colour	Size (mm)	Colony shape	Colony margin	Elevation	Sporulation	Pigments	Constituency	Region obtained
C2	Grey	3	Circular	Smooth	Raised	Grey	Brown	Powdery	CS
C3	Brown	1	Circular	Smooth	Raised	-	none	Leathery	CS
C5	Pinkish white	1	circular	smooth	Raised	pink	None	Leathery	CS
C8	Pink	1	Circular	Smooth	Raised	Pink	Pink	Leathery	DS
C10a	Grey	3	Circular	Smooth	Raised	Grey	Brown	Powdery	DS
C10b	White	1	Irregular	Smooth	Flat	White	yellow	Powdery	CS
C22	Grey	1	circular	Smooth	Raised	Grey	yellow	Powdery	DFZ
C25	Brown	3	circular	Smooth	Raised	Brown	-	Leathery	DFZ
P4i	Grey	2	circular	Smooth	flat	cream	Brown	Powdery	IS
C33	Brown	2	circular	smooth	raised	brown	brown	Leathery	DFZ
C34	White	3	irregular	smooth	raised	white	none	Leathery	DS
C43	Light yellow	3	irregular	rough	raised	white	None	Powdery	DS
C45	Light-green	1	Irregular	serrated	flat	white	green	Powdery	CM2
C52	light green	2	Circular	smooth	raised	white	none	Leathery	CM2
P1b	Light brown	1	Irregular	rough	flat	white	none	Powdery	DS
P1n	Brown	2	Circular	Serrated	Flat	Brown	Brown	Leathery	DS
P4x	Orange	2	Irregular	Rough	Flat	orange	Orange	Leathery	DS
L5	Yellow	1	Circular	Smooth	Flat	yellow	Yellow	Powdery	CM2
L6	Light green	1.5	Circular	Smooth	Raised	white	Green	Powdery	CM1
L14	Cream yellow	1	Regular	Rough	Flat	cream	None	Leathery	DS

NOTE: Symbols used represented media used; C=Starch Casein Agar; P1 and P4=International Streptomyces Project 1 and 4 respectively, L= modified Luria Bertani. Regions where isolates were obtained: CS= cultivated soil; DS=Dumpsite (urban); DFZ=degraded forest zone; CM1=Cattle manure; IS=Intact soil (forest zone); CM2=chicken manure

UNDER PEER REVIEW

3.2 Biochemical characterization of Actinomycetes and Gram stain test

The Gram staining analysis of the Actinomycetes isolates revealed that the selected isolates were gram-positive bacteria, as seen by the purple staining (Plate 2). Further analysis of the colonies indicated that the Actinomycetes isolates exhibited a wide range of microscopic features. The colonies' hyphal structure varied significantly, with some colonies displaying highly intertwined filamentous hyphae. Additionally, short filaments were observed in some colonies (Plate 2).

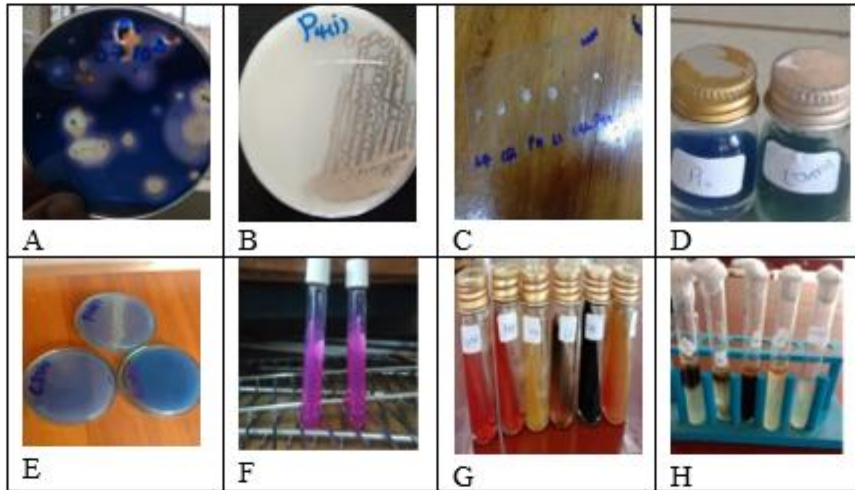


Note: Media used were denoted with; C=starch casein agar; LB= Luria Bertani, ISP= International Streptomyces project-1and 4; Isolates were coded based on media they were isolated from; P4=ISP-4; P1=ISP-1, L=LB, and C= starch casein. Land use systems; IS=Intact soil (forest zone); DFZ= degraded forest zone; CM1= cattle manure; CM2= chicken manure; AL= Agricultural land (cultivated soil)

Plate 2: Colony and microscopic characteristics of selected *Actinomycetes* isolate at 400X magnification on compound microscope.

Biochemical characteristics showed that all isolates hydrolysed starch and tested positive for amylase test while those positive for casein and catalase were at (77%); lipase test (57%); and citrate test (22% (Table 3). Plate 3 displays biochemical characters of some isolates.

UNDER PEER REVIEW



Note: A= starch hydrolysis test, B= Casein test, C= catalase test, D= Citrate test, E= lipase test, F= Urease test, G=Tripple iron sugar, H= Indole motility

Plate 3: Biochemical tests for active Actinomycetes isolates

Table 3: Biochemical characterization of Actinomycetes Isolates on Enzyme hydrolysis and

	C3	C8	C52	C10a	C10b	C42	C45	C34	C43	C50	C25	L6	L14	P1b	P4i	P4x	C26
Hydrolysis test																	
Casein	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
Amylase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	++	++	-	+	++	+	-	-	+	++	+	++	-	++
Lipase	-	-	+++	++	+	+++	-	-	-	-	+	-	-	+	++	-	-
Urease	Y	P	P	Y	P	P	P	P	Y	P	Y	P	P	Y	P	P	Y
Bt	Y	P	Y	P	P	Y	P	Y	P	P	Y	Y	Y	Y	P	P	Y
Cellulase	-	-	-	-	+	+	-	+	-	+	-	-	-	+	-	-	-
Citrate	-	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-	+
Sulfur	+	-	+	+	-	-	+	+	-	+	+	+	+	-	-	-	+
Indole	+	-	-	+	+	-	-	+	-	-	-	-	-	+	-	-	-
Motility	+	-	+	+	-	+	-	+	+	+	+	+	+	-	+	+	+
MR	-	-	-	-	-	++	++	+	-	++	+	+	-	-	+	+	+
VP	-	-	-	++	-	++	++	-	-	++	-	+	++	-	-	-	++
TSI-A	+	+	+	+	+	-	+	-	-	+	+	+	+	-	-	+	+
B	-	+	+	-	+	-	+	-	-	+	-	-	-	-	-	-	-
Sl	Y	P	P	Y	P	Y	Y	P	Y	P	Y	P	Y	Y	P	P	Y
Bt	Y	Y	Y	Y	P	Y	P	Y	Y	Y	Y	P	Y	Y	Y	Y	P
Carbon source																	
Glucose	+/-	+/-	+	+/-	+,g	-	+	-	-	+	+	+,g	-	+/-	-	+,g	+,g
Sucrose	-	-	+	-	+,g	-	+	-	-	-	+	+,g	+	+	-	+	+
Lactose	+	+	+	+	-	+/-	+	-	+	-	+	+,g	+	+	-	-	+
Fructose	+,g	+	+	+	+	+	+/-	-	+,g	+	+/-	-	-	+	-	-	+
Maltose	-	-	+	+,g	+	-	-	-	+	+	-	-	+,g	-	-	-	+
Dextrose	-	-	+,g	+	+	+	-	-	-	-	+,g	+	+	+,g	-	+	+

Notes: +++ = strongly positive; ++=moderately positive; += positive; +/- = doubtful; - = negative; g=gas production; Y=yellow; P=pink; Sl=slant; Bt= butt. Symbols representing media used; C=Starch Casein Agar; P1 and P4=International Streptomyces Project 1 and 4 respectively, L= modified Luria Bertani, MR= methyl red test VP, TSI=Triple iron sugar.

UNDER PEER REVIEW

3.3 Antibacterial activity of selected Actinomycetes

In the present study, active Actinomycetes isolates demonstrated the ability to inhibit the growth of the test bacteria, as evidenced by the presence of halo zones (Plate 4).

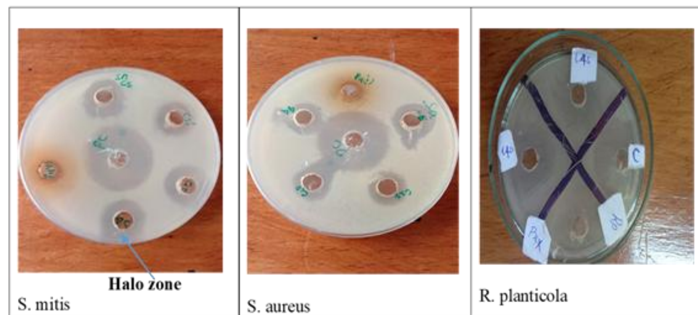


Plate 4: Secondary screening of isolates C5, C25, P4i and C45 showing antibacterial activity against *Streptococcus mitis*, *Staphylococcus auerus* and *Raoutella planticola*.

The mean diameter of the studied isolates for antibacterial activity against *Staphylococcus aureus* ranged from 6.5 mm to 12.25 mm. Isolate C52 (12.25 mm) demonstrated the most antibacterial activity, while isolate C25 (6.5 mm) demonstrated the least inhibitory activity against *S. aureus*. All of the isolates tested positive for antibacterial activity against *S. aureus* (Table 4).

The mean inhibition zone for *Escherichia coli*, ranged from 6.25 to 8.75 mm. Isolate L3 had the highest inhibitory activity against *Escherichia coli*, with a value of 8.75 mm. There was a statistically significant difference (P =.05) between antagonistic isolates and *Listeria monocytogenes*. When compared to the standard, streptomycin (16.3 mm), isolate L6 demonstrated the highest antagonistic activity of 16.23 mm against *Listeria monocytogenes. Listeria monocytogenes* showed resistance to isolates P1b, L3 and L14 (6.0 mm) (Table 4).

Isolates C52, L14, C43, L6, and C8 had the highest activity against *Staphylococcus aureus*, *Raoutella planticola*, *Vibrio furnissii, Listeria monocytogenes,* and *Streptococcus mitis*, respectively. These isolates were isolated from; chicken manure (C52), dumpsite soil (L14, C43, C8), and cultivated soil (L6). The greatest efficacy against *E. coli* was demonstrated by isolate L3 isolated from intact soil (forest zone), followed by isolate C3 isolated from cultivated soil.

UNDER PEER REVIEW

Table 4: Zones of inhibition (mm) of the selected pathogenic microorganisms in a secondary screening of the Actinomycetes isolates

		Zone of inhibition (mm)					
Isolates	LUS	Sa	Sm	Ec	Lm	Vf	Rp
CT(strept)		15.67a	16.67a	16.00a	16.33a	16.00a	16.00a
C52	CM2	12.25b	10.00bcd	6.00d	11.00cde	8.00fghi	12.50cd
L14	DS	11.50bc	10.75bc	6.00d	6.00i	8.00fghi	12.50cd
C8	DS	11.25bcd	11.25bc	7.50cd	8.75ghi	12.00bc	11.00cd
P4x	IS	10.50bcde	9.25bcdefghi	7.50cd	10.25defghi	6.00i	12.50cd
P4i	IS	10.50bcde	9.75bcdefg	6.00d	11.50cd	6.00i	13.00bcd
C33	DFZ	10.50bcde	8.00cdefgh	7.50cd	7.50hi	6.70h	6.75h
C5	CS	10.00bcdef	6.25fgh	6.75cd	12.50bc	6.75hi	6.00j
C28	CM2	10.00bcdef	8.75bcdefgh	7.50cd	12.50bc	9.00fgh	9.00cdefgh
C45	CM2	10.00bcdef	6.00f	7.25cd	9.50efgh	9.50efgh	8.50cdefgh
C43	DS	9.50bcdefg	9.75bcdefgh	8.25bcd	11.50cd	11.50bcd	11.50cdef
C50	DFZ	9.50bcdefg	7.25efgh	6.00d	12.50bc	11.50bcd	15.50a
C22	DFZ	9.50bcdefg	11.50b	7.75cd	9.00efgh	9.75cdefg	13.00bcd
C26	DS	9.50bcdefg	7.75efgh	7.75cd	7.50hi	11.25bcd	12.25cde
C61	DS	9.00cdefg	7.75efgh	6.50cd	11.75bcd	8.75efgh	9.75cdefgh
C3	CS	9.00cdefg	8.75bcdefgh	8.25bcd	7.00i	10.50bcde	8.75cdefghi
L6	CM1	8.75cdefg	8.25bcdefgh	6.00d	16.23a	8.00cdefgh	10.50defg
C40	CM1	8.75cdefg	7.00defgh	7.00cd	10.50defg	12.50b	12.25cde
P4ii	CM2	8.50cdefg	7.75defgh	6.25d	7.50hi	10.75bcde	8.75cdefghi
L14	DS	8.25defg	9.00bcdefgh	7.50cd	11.00cde	6.00i	13.25ab
C10b	CS	8.25defg	7.75defgh	6.75cd	8.75ghi	9.75cdefg	8.75cdefghi
C27	CM2	8.25defg	6.00f	6.00d	6.00i	11.25bcd	6.00j
C10a	DS	8.25defg	7.50defgh	8.00bcd	7.00i	10.50bcde	11.00cdefg
C46	DS	8.00efg	9.50bcdefg	8.00bcd	9.50efgh	6.00i	11.00cdefg
C30b	IS	7.75efg	6.25fg	7.00cd	7.50hi	7.75ghi	7.75hij
L3	IS	7.75efg	8.75bcdefgh	8.75bcd	6.00i	10.00bcdef	9.75cdefgh
C2	CS	7.50efg	11.25bc	10.25b	9.00efgh	6.00i	6.00j
P1b	DS	7.25fg	6.25fg	7.70cd	6.00i	10.0bcde	9.25cdefgh
C34	DS	7.00fg	8.50bcdefgh	6.00d	11.50cd	6.00i	6.00j
L1	CM1	6.75g	7.00defgh	7.00cd	13.25b	11.50bcd	7.50hij
C25	DFZ	6.50g	6.50efh	6.00d	10.25defg	6.00i	9.00cdefghi
Mean		9.16	8.54	7.37	9.79	9.11	10.07
LSD		3.13	8.53	2.46	1.72	2.02	2.74
CV (%)		24.16	27.07	23.58	12.34	15.72	19.29

aMeans followed by the same letters are not significantly different at 5% probability level.
NOTE: Test organisms: Ec= *Escherichia coli*; Lm= *Listeria monocytogenes*; Vf= *Vibrio furnissii*; Rp= *Raoutella planticola*; Sa= Staphylococcus *aureus and Sm= Streptococcus mitis. Media used represented as C=starch casein, L=Luria Bertani*, P1 and P4=ISP-project media. Land use system (LUS) where isolates were obtained: AL=agricultural (cultivated) land; DS=Dumpsite (urban); DFZ=degraded forest zone; CM1=Cattle manure; IS=Intact soil (forest zone); CM2=chicken manure

3.4 Molecular characterization of bioactive *Actinomycetes* isolates

The 16s rRNA sequence analysis revealed that eight isolated strains, C10a, C10b, C3, C8, P41, P4xiv, P1b and C45 belong to the *Streptomyces* species. The 16s rRNA gene sequences of the isolated Actinomycetes in this study were aligned with sequences with similarity indices above 97% retrieved from the NCBI gene bank (https://www.ncbi.nlm.nih.gov), which were then identified up to species or genus level (Table 4). The Neighbor Joining phylogenetic tree (Figure 1) built from the combined 16S rRNA gene sequences showed that isolate P4 (Ac No. ON398794.1) was clustered with *Streptomyces celluloflavus* (Acc No. JQ0066794.1 and Ac No. OK56094.1) from the

gene bank at 84% bootstrap support value. Isolate C45 (Accession No. ON398796.1) was identified as *Streptomyces griseobrunneus* (Accession No. KP137823.1 and Accession No. KF703725.1) at similar bootstrap support values. Isolates P4x and C8 (Ac No. ON398793.1 and Accession No. ON398799.1 respectively) were both clustered at 100% bootstrap support with *Streptomyces pratensis* (Ac Nos. OM909122.1 and OK148886.1). Isolate P1b (Ac No. ON398795.1) was identified as *Streptomyces crystallinus* and was clustered with strains NBRC 15401 (NR_041177.1) and CSSP421 (NR_115376.1) at 100% bootstrap support. Isolates C10b, C10a and C3 (Ac No. ON398797.1, ON398798.1 and ON398800.1) clustered with *Streptomyces eurocidicus* strains DLHG12 (HQ844449.1) and IIL-Asp 72 (KX380889.1) at 99% bootstrap.

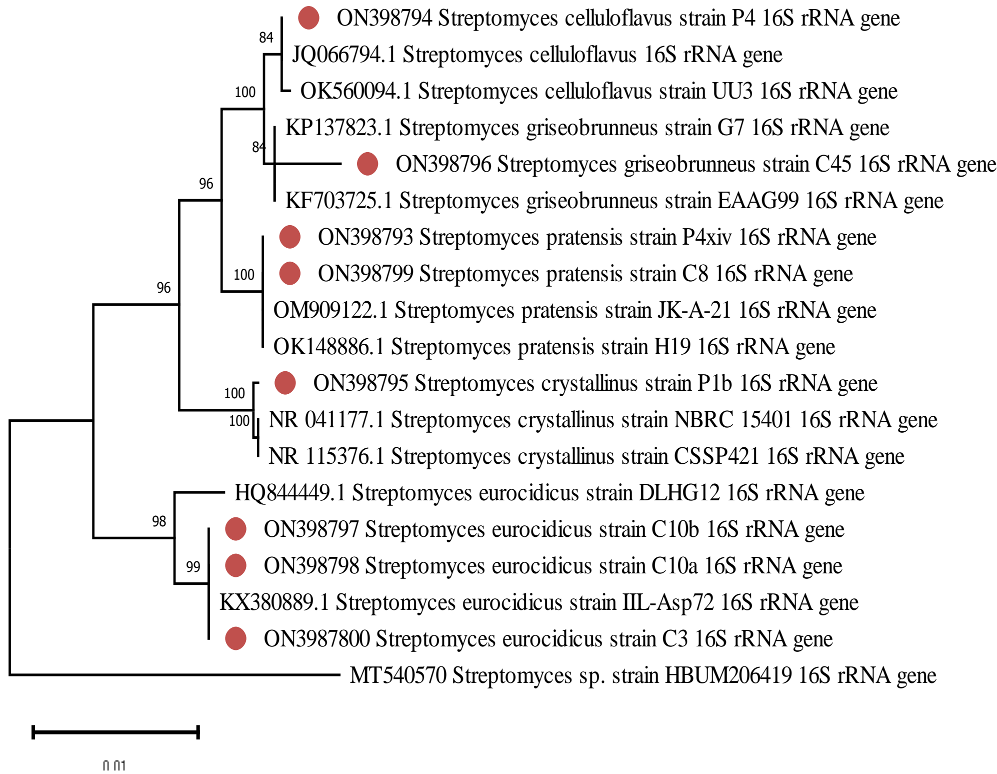


Figure 2. ***A Neighbor-Joining phylogenetic tree drawn from 16S rRNA gene sequences of Streptomyces species isolated from soils from Meru South and other similar sequences retrieved from the NCBI database. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Tamura et al., 2004). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree***

Table 5: Identities and accession numbers of 5 *Streptomyces* isolates obtained from different Land Use Systems in Meru South Sub-County, based on 16S rRNA gene sequences and similar sequences obtained from NCBI

Isolate Identity	NCBI Acc. No. (this study)	Site of isolation	BLAST similarity %	Species Identity	Similar NCBI Acc. No.	Country of origin
P4i	ON398794.1	IS	100	*S. celluloflavus*	JQ0066794.1	-
C45	ON398796.1	CM2	100	*S. griseobrunneus*	KP137823.1	Iran
P4x	ON398793.1	Dumpsite	100	*S. pratensis*	OM909122.1	China
C8	ON398799.1	Dumpsite	100	*S. pratensis*	OK148886.1	China
P1b	ON398795.1	Dumpsite	100	*S. crystallinus*	NR_041177.1	Japan
C10b	ON398797.1	CS	100	*S. crystallinus*	NR_041177.1	Japan
C10a	ON398797.1	Dumpsite	100	*S. eurocidicus*	HQ844449.1	China
C3	ON398797.1	CS	100	*S. eurocidicus*	KX380889.1	Brazil

Note: CM2= chicken manure; CS= Cultivated soil; IS= Intact soil; *S= Streptomyces*

4. DISCUSSION

The consistent circular form of the colonies, with round margins and varying elevations across different agar media revealed in the present study shows that these morphological traits may be inherent to Actinomycetes growth patterns, presumably affected by their filamentous nature. The varied variety of colony colours seen in the current study indicates Actinomycetes' variability at the species or strain level. The morphological traits obtained in the present study are consistent with the findings of Smith et al. [50], who discovered a wider variety of colony colours in Actinomycetes isolated from agricultural soils than those isolated from forest soils. In the current study, the aerial mycelium ranged in colours from white to grey to powdery, leathery, chalky, cream yellow, pink, and purple. The substrate mycelium colours ranged from pink, yellow, green, black, red to brown. The findings of this study are consistent with the findings reported by Jeffery [51], who revealed that substrate mycelium was brown to orange in colour while aerial mycelium was white, creamy, chalky, powdery, brown, grey, and pink in appearance. Furthermore, Johnson et al. [52] revealed significant variations in spore production patterns and mycelial structures across Actinomycete isolates isolated from grasslands and agricultural fields.

The Actinomycetes isolates examined in the current study were gram-positive bacteria, as seen by the purple staining. This is because during decolourization process, the thick peptidoglycan coating in the cell walls of gram-positive bacteria attracts the crystal violet stain. This finding is consistent with study findings reported by *Smith et al*. [50], in the study conducted on identification of Actinomycetes as Gram-positive bacteria in practical Handbook of Microbiology. In the present study some of the colonies possessed a dense and intricate network of filamentous hyphae that were extensively interwoven. The hyphal structure exhibited by the colonies varied substantially among the isolates in the present study. This finding suggests the presence of Actinomycetes strains capable of forming complex mycelial networks. Several colonies also showed branching hyphae, which relates to Actinomycetes' growth of aerial mycelia. Additionally, several colonies in the current study had short filaments. Short filaments may indicate a significant variation in colony morphology or may be related to certain genetic or environmental factors influencing colony growth in some isolates [53]. The observed morphological diversity of the Actinomycetes colonies, ranging from highly intertwined filamentous hyphae to branching hyphae, is in consistent with the known characteristics of this bacterial group [54].

UNDER REVIEW

The urease test results showed that some isolates showed a deep pink colour in both the slant and butt on the urea agar slant, indicating a positive result for urease production. This deep pink colour indicates alkaline conditions brought on by ammonia synthesis. Other isolates, on the other hand, showed a pink butt and a yellow slant, indicating a lack of urease activity. Some isolates in the current study tested negative for the Indole test, indicating that no Indole was produced. The clear zone observed on skim milk agar plate indicated bacterial growth (positive test) for casein hydrolysis activity. This indicated ability of isolates to degrade casein protein due to production of exoenzyme proteinase (caseinase). The catalase test resulted in a positive reaction (the appearance of bubbles), indicating that catalase had broken down the hydrogen peroxide and produced oxygen gas as a result. The ability of the isolate in the current study to use sodium citrate as the only source of carbon and inorganic ammonium phosphates as the source of nitrogen was demonstrated by the medium's change of colour from green to blue during the citrate test.

The phenol red's colour changed from red to yellow, indicating the conversion of sugars, revealed the ability of isolates to utilize carbon sources. The Durham tubes' ability to produce gas indicates fermentation activity in the isolates that were examined in the current study. The isolates showed the fastest rate of growth on isolates cultivated with 1% glucose and lactose out of the five different carbon sources examined in the present study, indicating high metabolic versability of the carbon source. Li et al. [43]) in his study observed a wide range of carbon-source utilization patterns among Actinomycetes isolated from grapevine rhizosphere soil.

The findings of the antibacterial activity tests in the present study revealed that the isolated Actinomycetes isolates were more effective against gram-positive bacteria than gram-negative bacteria. These results are consistent with earlier research conducted by Gebreyohaness et al. [25], who found that Actinomycetes strains had antibacterial properties against a range of bacterial strains in their study on the isolation and characterization of potential antibiotic-producing Actinomycetes from water and sediments of Lake Tana, Ethiopia. However, a number of the isolates showed only limited effectiveness against *E. coli*, in the current study which is similar with the findings of Oskay et al.[55] on study report on the antibacterial activity of a Actinomycetes isolate from Turkish agricultural soils.

Streptomyces celluloflavus, *S. griseobrunneus*, *S. pratensis*, *S. crystallinus*, and *S. eurocidicus* were the five *Streptomyces* species identified during the current study. This finding is in agreement with study report by Nonoh et al. [56], which identified *Streptomyces* species after molecular characterization of soil Actinomycets from soil samples collected within a protected area in Kenya. However, the current study findings differs from that of Edemekong et al. [57] which revealed different genera of soil Actinomycetes, namely; *Rhodococcus*, *Leucobacter*, J*atrophihabitan*, and *Nesterenkoniamas* identified from soil samples in Calabar metropolis, Nigeria. The present study did not identify a wide variety of Actinomycetes. Other isolates' lack of amplification could be attributable to their belonging in rare Actinomycetes genera, implying that future investigations should investigate employing a broader range of primers, including genus-specific primers. The current study's 16S rRNA gene phylogenetic analysis indicated that these isolates were Actinomycetes strains, with similarity scores ranging from 84% to 100%.

According to NCBI-BLASTn study, *Streptomyces celluloflavus* and the P4i isolate shared a sequence similarity of 84%. The species has been recognized for its capacity to produce aurethricin, a compound that has the ability to enzymatically break down cellulose. By producing poly amino acids, it also has significant effects on the pharmaceutical industry. The

UNDER PEER REVIEW

sequences of the isolates C10a and C3 were 99% similar to *Streptomyces eurocidicus*. This specific type of strain has the capacity to produce 2-nitroimidazole, Azomycin, eurocidin C, D, and E. *Streptomyces crystallinus* and isolates P1b and C10b had an identical 100% sequence similarity. *Streptomyces crystallinus* is well-known for producing hygromycin-B, an aminoglycoside antibiotic that kills bacteria, fungi, and higher eukaryotic cells by interfering with protein synthesis [58]. It also poduces Hygromycin A (HA) that has antibacterial properties due to its capacity to inhibit protein synthesis via a methylenedioxy bridged-aminocyclitol structure. A study report by Bhandari et al. [59] identified *Streptomyces* species, namely S. species_SB1, S. species_SB3, and S. species_SB10, that showed potential antibacterial characteristics in their study on the characterization of antibiotic-producing *Streptomyces* species from untouched habitats in Nepal.

5. CONCLUSION AND RECOMMENDATION

The morphological analysis done in the present study provided valuable insights into the colony morphology, spore arrangement, and pigmentation of the isolated Actinomycetes, enabling preliminary identification and differentiation of the species. In secondary screening, the Actinomycetes crude extracts demonstrated substantial antibacterial activity against the selected test pathogens. This shows that these extracts contain bioactive compounds with potential antibacterial activity. The isolated Actinomycetes show promising antibacterial activity against the test pathogens. As a result, the study recommends continued research into Actinomycetes as an important resource to develop novel antibiotics.

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