library@chuka.ac.ke;www.chuka.ac.ke

ISOLATION AND CHARACTERIZATION OF ACTINOMYCETES FROM LAND USE SYSTEMS IN MERU SOUTH SUB-COUNTY, KENYA AND SCREENING THEIR ANTIBACTERIAL ACTIVITIES

Gitari J. M., Muraya M.M., Onyango B. O. and Maingi J. M.

Department of Biological Sciences, Chuka University, P.O. Box 43844-00100, Chuka, Kenya.

Department of Plant Sciences, Chuka University, P.O. Box 43844-00100, Chuka, Kenya

Department of Biological Sciences, Jaramogi Oginga Odinga University of Science and Technology, P.O. Box 210- 40600, Bondo, Kenya.

Department of Biochemistry, Microbiology and Biotechnology, School of Pure and Applied Sciences, Kenyatta University, P.O. Box 43844 - 00100, Nairobi, Kenya

Citation:

Gitari J. M., Muraya M.M., Onyango B. O. and Maingi J. M. (2023). *Isolation and Characterization of Actinomycetes from Land Use Systems* in Meru South Sub-County, Kenya and Screening Their Antibacterial Activities. In: Isutsa, D. K. (Ed.). *Proceedings of the Chuka University 9th Annual International Research Conference held in Chuka University, Chuka, Kenya from 24th to 25th November*, 2022. 409-421 pp.

ABSTRACT

To combat the growth of multidrug resistance to existing antibiotics, the quest for novel bioactive compounds from Actinomycetes in virgin environments has become increasingly crucial. In this study, Actinomycetes were isolated, characterized, and tested for antibacterial activity from the land use system in the Meru South sub-county, Eastern Kenya. Using morphological, biochemical, and molecular techniques, Actinomycetes were described after being cultivated on Starch Casein Agar, Luria Bertani (M1), and International Streptomyces Project Medium (ISP-1 and ISP-4). The isolates were tested for their ability to combat the following six pathogenic bacterial strains; Staphylococcus aureus (ATCC 25923), Listeria monocytogenes (NCTC 11994), Streptococcus mitis (NCTC 12261), Escherichia coli (ATCC 25922), Vibrio furnissii (NCTC 11218), and Rauotella planticola (NCTC 19528). Further identification of potential isolates was done based on 16S rRNA gene sequence analysis. Phylogenetic analysis was done using Molecular Evolutionary Genetics Analysis (MEGA X), and a phylogenetic tree constructed using the

Neighbor-Joining methodThe results for morphological characters showed that isolates' morphology colours ranged from grey to purple to pink to brown to yellow to red to cream- white. The biochemical examination revealed that the majority of them were gram-positive, capable of hydrolyzing starch, urea,casein, and lipase. Few isolates were positive for the cellulose and catalase test. The antibiotic activity of the Actinomycetes isolates against Listeria monocytogenes varied significantly (p=0.05) among them. In comparison to standard streptomycin (16.3mm), isolate L6 (16.23mm) demonstrated the strongest antagonistic activity against L. monocytogenes, followed by isolates C50 (15.5mm) and C52 (12.25mm) against Raoultella planticola. In the phylogenetic tree created using the combined sequences and neighbour joining, isolate P4 was shown to be associated with Streptomyces celluloflavus, isolate C45 with Streptomyces griseobrunneus, isolates Pxiv and C8 with Streptomyces pratensis, isolate P1b with Streptomyces crystallinus, and isolates C10b, C10a, and C3 (Streptomyces eurocidicus). This study shows soil Actinomycetes obtained from land use system in Meru South sub-County has antibacterial activity and may be useful in biomedical applications.

Keywords: Soil Actinomycetes, 16SrRNA, Antibacterial Activity

INTRODUCTION

Due to the increased burden of multidrug resistance, there has been increasing interest in researchers searching for novel bioactive secondary metabolites to overcome the multidrug resistance of pathogens (Malison et al., 2020). The possible way to access new antibiotics is to find new bacteria and fungi isolating approaches and to search for new sites or environments for selection of microbes (Frieri et al., 2017). Soil is an important natural source for many microorganisms including Actinomycetes which produce many bioactive natural resources including clinically important drugs (Khebizi et al., 2018). Actinomycetes are Gram-positive microorganisms widely distributed in nature (Barka et al., 2016). The most dominant genus within Actinobacteria is Streptomyces belongs to the family Streptomycetacea which are highly abundant in different ecological niches (Hamid et al., 2020). Streptomyces genus contains Gram positive bacteria, having guanine cytosine (GC) rich (70%) genetic material (Cetinkaya, 2021).

Actinomycetes have more genes that encode for various enzymes that promote the production of bio-active secondary metabolites (Xia et al., 2020). Antibiotics are the most vital bio-active secondary metabolites for the treatment of infectious diseases. Since the last two decades, multidrug resistance (MDR) increased due to the absence of new antibiotics that is a basic challenge for effective treatment of infectious diseases (Ouchari et al., 2019). A continuous screening of potential bacterial taxa for production of secondary metabolites is crucial for discovery of novel compounds (Zothanpuia et al., 2017).

Molecular characterization of Actinomycetes for the production of secondary metabolites offers a strong research perspective. For the identification of novel strains, 16S rRNA sequence method is most prevalently used due to its presence in almost all bacteria and the function of 16S rRNA gene over time has not changed (Jand and Abbott, 2007). Analysis of 16S-rRNA gene sequence amplification has been

demonstrated to be a potential method for microorganism's phylogeny investigation. It is reported that 16S rRNA gene sequencing provides genus identification in most cases more than 90%

(Guo et al., 2008). The current research study was designed to assess Actinomycetes for secondary metabolites production and also to characterize selected Actinomycetes using morphological, biochemical and molecular means.

MATERIALS AND METHODS

Study Site

The study was conducted using soil samples collected from Mt. Kenya forest (S0° 18.247' E37° 34.111'), farm lands (S0° 19.347' E37° 39.483') and solid waste dump site in Chuka (S0° 19.699' E37° 39.176') Sub County in Tharaka-Nithi County in Kenya. Tharaka-Nithi County lies between latitude 000 07' and 000 26' South and between longitudes 37 0 19' and 37 0 46' East with an elevation of 600–1500m (above sea sea level). Meru south sub- County is characterized by low and erratic rainfall as well as high evapotranspiration rate (Jaetzold et al., 2007). The bimodal rainfall ranges from 1200-1400mm and experiences temperatures range between 24o C and 26o C (Omondi et al., 2014). Majority of the smallholder farmers in these areas depends on rain-fed agricultural production system (Mugwe et al., 2009) and major crops in the area are; Tomatoes, maize, mangoes, sorghum among others. The predominant soil type is humic nitisols, typically deep and weathered soil with moderate to high fertility while the soil texture is predominant sand clay loam (Ngetich et al., 2014).

Soil Sampling

Soil samples were randomly collected from six different land uses. The soils were collected along a transect using zigzag soil collection technique. Soil collection regions included; uncultivated soils within the Mt. Kenya forest, cultivated soils outside Mt. Kenya forest, farm yard manure from Chuka University and soils from solid wastes from the dumpsite in Chuka town. Soil collection was carried out using spiral soil auger which was cleaned with 10% formalin to avoid cross- contamination of soil samples. The soils collected were labelled, packed in sterile ziplock bags and transported to Chuka university for further processing.

Pretreatment of soil samples

The composite soil samples were spread out on aluminium foil on the laboratory bench and allowed to dry for 4-6 days at room temperature, to decrease moisture-dependent bacteria and fungus (Zainal et al., 2016). The air dried soil sample were crushed to make fine powder soil sample. To effect the treatment, 0.1 g $CaCO_3$ was added to 10g of fine soil sample and subjected to hot air oven at 120 ºC for one hour. (Messaoudi et al., 2015; Zainal et al., 2016.

Isolation Actinomycetes from soil samples

One gram (1 g) of composite soil samples each from different land uses were separately added to a test tube containing 9 ml saline water and shaken vigorously at room temperature (25 ± 2°C), using an orbital shaker at 200 rpm for 10 min. The test tubes were considered as stock culture for the soil samples from which serial dilution preparations were made. Aseptically, 100 µl aliquot from the stock solution was transferred to a test tube containing 9 ml of sterile physiological saline and mixed well. From these test tubes, 100 µl of aliquot was again transferred and mixed with another 9 ml of distilled water to make 10-2 dilution factor. Similarly, dilutions up to 10-4 were made using serial dilution technique for all soil samples (Sujatha and Swethalatha, 2017).

After serial dilution, 0.1 ml of each sample was separately plated using spread plate technique in Luria Bertani Agar (M1), Starch Casein Agar and International Streptomyces Project (ISP-1) and (ISP-4) media. The plates were incubated at 28°C and observed from 5th day onwards for 7 days. After incubation, Actinomycetes isolates were distinguished from other microbial colonies by characteristics such as tough, leathery colonies which are partially submerged into the agar (Yaminisudha et al., 2015).

Phenotypic Characterization of Actinomycetes Isolates

Morphological characteristics of the selected pure isolates were studied by inoculating them into starch casein agar (Yang et al., 1995). Morphological characteristics such as colony characteristics, pigment production, presence or absence of aerial and substrate mycelium were characterized based on guidance by Shrilling and Gottlieb (1966). The gram stain test was carried out with the method described by Pooja et al., 2015.

Biochemical Characterization of Actinomycetes Isolates

The ability of the isolates to utilize D-Glucose, D-Fructose, sucrose, maltose, dextrose and lactose was tested. The isolates were mixed (1% w/v) with the basal medium followed by incubation at 30°C for 7 days (Pooja et al., 2015). The proteolytic activity of selected isolates was studied using milk casein agar. The isolates were streaked on milk casein agar and incubated at 27 °C for 5-7 days. This loss of opacity was the result of a hydrolytic reaction yielding soluble, non-colloidal amino acids, and it represents a positive for casein hydrolysis (Salle, 1948).

Isolates were streaked on starch agar plates for the amylase test and incubated at 27 °C for 5-7 days. Then an iodine solution was poured all over the plates (Salle, 1948). Tryptophan’s synthesis and the ability to generate hydrogen sulphide from sources such organic Sulphur and sulfur-containing amino acids were examined using Sulfur-Indole Motility (SIM) agar medium. Kovac's reagent was added to 48-hour cultures of each isolate to identify the presence of indole (Harold, 2002). After incubation, the medium lacked a black tint, which meant there was no hydrogen sulphide present (Cappuccino & Sherman, 2002).

Methyl red and Voges Proskaeur tests were carried out with the method described by Prescott et al. (1991). Catalase production was determined by the addition of 3% hydrogen peroxide to Tryptic Soy Agar (TSA) cultures of each isolate based on the methods outlined by Cappuccino and Sherman (2002). A positive reaction was indicated by the formation of bubbles, which indicated production of catalase activity.

The citrate test is used to show the ability of a microorganism to utilize citrate as the sole carbon source (Harold, 2002). The utilization of citrate depends on the presence of an enzyme citrase produced by the organism. Isolates were also streaked on Simon's citrate slant agar and incubated at 30 °C for 7 days.

Screening of selected Actinomycetes for antagonistic activity against test pathogens Primary Screening of Antimicrobial of Actinomycetes Isolates

The primary screening was done according to Bizuye et al., 2013; Zainal et al., 2016. The antibacterial activities of the isolates were determined on Starch casein agar media by the perpendicular streak method (Egorov, 1987). The recovered Actinomycetes isolates were cross-streaked as a single line on solidified starch casein agar media in a petri-dish and were incubated at 28°C for three days. The test organisms were then cross-streaked perpendicular to the original streak of isolates. The antibacterial activities of different cultures of Actinomycetes was determined against the bacterial strains which include; Escherichia coli (ATCC 25922), Listeria monocytogenes (NCTC 11994), V. furnissii (NCTC 11218), R. planticola (NCTC 19528), Staphylococcus aureus (ATCC 25923), and Streptococcus mitis (NCTC 12261).

The test bacterial suspensions preparation and turbidity standardization was done according to Ataee et al. (2012). The suspension from a test pathogen was taken using a sterile inoculating loop and it was streaked perpendicular to the Actinomycetes isolate on starch casein plate media. These were incubated at 37º C for 24 hours for antibacterial activity observation. The antibacterial activities of isolates against test pathogens were observed by visual detection and the inhibition zone (antibacterial activity) was measured using the graduated ruler. Control plates of the same medium without Actinomycetes growth were also simultaneously streaked with test organism to study their normal growth.

Secondary Screening of Actinomycetes Isolates for Antibacterial Activity

The isolates showing positive results in primary screening were inoculated using the submerged fermentation procedure in boiling tubes containing 30 ml of sterile medium starch casein broth. The inoculated boiling tubes were incubated on rotary incubator shaker (Biotechnich India) at 28°C and 150 rpm for 7 days. The fermentation broth was centrifuged (Remi, RM12C, India) at 10,000 rpm and 4 °C. The supernatant was analysed for antimicrobial activity by disc method using the same test microorganisms used in primary screening (Pisano et al., 1986). 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 and 25 µl of Dimethyl sulfoxide (DMSO) as negative control and 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 millimetre in diameter (Mohanraj et al., 2011).

Isolation of genomic DNA

Isolates were inoculated in the starch casein agar media and incubated at 28°C for 7 days. Isolation of genetic deoxyribose nucleic acid (gDNA) was carried out using standard phenol-chloroform methods (Gumińska et al., 2018). For the identification of bacterial 16S rRNA sequence analysis was performed using universal primer 27F (5'- AGAGTTTGATCCTGGCTCAG-3') and 1429R (5'-GGTTACCTTGTTACGACTT-3'). The polymerase chain reaction (PCR) condition was set at initial denaturation at 94°C for 5 min; 30 cycles at 95°C for 30 secs, 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 were analyzed by 0.7% agarose gel electrophoresis. The purified PCR products were sequenced using 27F and 1429R primer with Sanger sequencing methods at Genotech.

Sequence analysis

The 16S rRNA sequence of the isolates were deposited in the National Center for Biotechnology Information (NCBI) gene bank. The homology search of the partial DNA sequence of 16S rRNA of the isolates was performed using the NCBI

database and multiple sequence alignment was performed using ClustalW and the phylogenic tree was plotted using MEGA version 6 software by the neighbor-joining methods with bootstrap values calculated from 1,000 replications.

Statistical analysis

Data collected on the phenotypic characteristics of the Actinomycetes isolates that included growth characteristics, colony colour, colony shape, margin type, elevation and sporulation was used to construct dendogram based on Euclidean distance in R studio. Data collected on Actinomycetes load from different areas (Land uses) were normally distributed and were therefore subjected to the analysis of variance (ANOVA) and significant means separated using Least Significant Difference at α=

0.05 in SAS version 9.4. The data collected and recorded on diameters of zones of inhibition on antagonistic effect of pure cultures of Actinomycetes against test organism was subjected to one- way ANOVA in secondary screening. The data was then separated with LSD) with descriptive analysis type on different isolates against different test organism cultures using SAS 9.4 software. The results with at α= 0.05 was considered to be statistically significant.

RESULTS

Morphological Characterization

The Actinomycetes generally were circular in shape with round margin and raised or flat elevation (Table 1). Table 1: Morphological characterization of Actinomycetes isolates

P1g	Light yellow	1.5	circular	smooth	raised	white	light yellow	Leathery	+
P1n	Cream yellow	2	irregular	rough	flat	cream	none	powdery	+
P4m	Cream-white	1.5	Circular	Smooth	Raised	White	None	Leathery	+
P4(I)	Brown	2	Circular	Serrated	Flat	Brown	Brown	Leathery	+
P4(ii)	Greyish red	1.5	Circular	Serrated		Flat	Grey Red	Powdery	+
P4xiv	Orange	2	Irregular	Rough	Flat	orange	Orange	Leathery	+
L5	Yellow	1	Circular	Smooth	Flat	yellow	Yellow	Powdery	+
L6	Light green	1.5	Circular	Smooth	Raised	white	Green	Powdery	+
L14	Cream yellow	1	Regular	Rough	Flat	cream	None	Leathery	+

The colour of morphology varied among isolates from grey, purple, pink, brown, cream, yellow, red, green-yellow and cream-white. Colony reverse side had brown, yellow, pink or grey in colour. Further, most colonies had hard textures that were difficult to scrap off the agar. Some of the colonies were also dry, chalky, leathery and powdery. The mycelium of Actinomycetes showed varied colour colony. Lastly, all colonies possessed an earthy smell. Dendogram constructed based on Euclidean distance classified the Actinomycetes isolates in three clusters and eight subclades (Figure 1).

Figure 1: Cluster membership of Actinomycetes isolated from different soils samples in Meru South Sub-County based on Euclidean distance clustering

Biochemical characterization of Actinomycetes

Most of the selected active Actinomycetes stained purple in gram stain, showing that they were gram-positive bacteria (plate 1).

ISPI A5

ISP1 D4a

Casein D4b

Casein E5a

casein E5b

Casein D15

Casein E5b

LB B4C

LB E1

ISP1 A1 b(ii)

ISP1 C8

Casein D13

Plate 1: Colony and microscopic characteristics of selected Actinomycetes isolate at 400x magnification on light microscope

Biochemical characteristics of the selected Actinomycetes isolates was presented in Table 2 showed that all isolates hydrolyzed starch. while most isolates were positive for catalase test except isolate C50, C25 and P4XIV. Isolates C26 and C52 fermented all sugars used. The colony and microscopic characteristics of selected Actinomycetes isolates are shown in plate 2. The colonies ranged from having highly intertwined filamentous hyphae to branching hyphae. In some isolates, short filaments were also seen.

Table 2: Biochemical characterization of Actinomycetes

	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

Antibacterial Activity of Selected Actinomycetes

In the present study, selected Actinomycetes isolated from soil samples were observed to demonstrate inhibitory activity against test strains Escherichia coli (ATCC 25922), Listeria monocytogenes (NCTC 11994), V. furnissii (NCTC 11218), R. planticola (NCTC 19528), Staphylococcus aureus (ATCC 25923), and Streptococcus mitis (Table 3).

Table 3: Zones of inhibition (mm) of the selected pathogenic microorganisms in a secondary screening of the Actinomycetes isolates

	Zone of inhibition(mm)										
Isolates	Test Organisms										
	Sa	Sm	Ec	Lm	Vf	Rp					
CT(strept)		15.67a	16.67a	16.0a	16.33a	16.0a	16.0a				
C52	12.25b	10.0bcd	6.0d	11.0cde	8.0fghi	12.5cd					
L14	11.5bc	10.75bc	6.0d	6.0i	8.0fghi	12.5cd					
C8	11.25bcd		11.25bc	7.5cd	8.75ghi	12.0bc	11.0cde				
P4ixv	10.5bcde		9.25bcdefghi		7.5cd	10.25defgi		6.0i		12.5cd	
P4i	10.5bcde		9.75bcdefg		6.0d	11.5cd	6.0i		13.0bcd		
C33	10.5bcde		8.0cdefgh		7.5cd	7.5hi	6.75hi		6.75ij		
C5	10.0bcdef		6.25fgh	6.75cd	12.5bc	6.75hi	6.0j				
C28	10.0bcdef		8.75bcdefgh		7.5cd	12.5bc	9.0fgh		9.0cdefghi		
C40	10.0bcdef		6.0f	7.25cd	9.5efgh	9.5efgh	8.5cdefghi				
C43	9.5bcdefg		9.75bcdefgh		8.25bcd	11.5cd	11.5bcd		11.5cdef		
C50	9.5bcdefg		7.25efgh		6.0d	12.5bc	11.5bcd		15.5a		
C22	9.5bcdefg		11.5b	7.75cd	9.0efgh	9.75cdefg			13.0bcd		

C26	9.5bcdefg	7.75efgh	7.75cd	7.5hi	11.25bcd	12.25cde
C61	9.0cdefg	7.75efgh	6.5cd	11.75bcd	8.75efgh	9.75cdefgh
C3	9.0cdefg	8.75bcdefgh	8.25bcd	7.0i	10.5bcde	8.75cdefghi
L6	8.75cdefg	8.25bcdefgh	6.0d	16.23a	8.0cdefghi	10.5defg
C40	8.75cdefg	7.0defgh	7.0cd	10.5defg	12.5b	12.25cde
P4ii	8.5cdefg	7.75defgh	6.25d	7.5hi	10.75bcde	8.75cdefghi
L14	8.25defg	9.0bcdefgh	7.5cd	11.0cde	6.0i	13.25ab
C10b	8.25defg	7.75defgh	6.75cd	8.75ghi	9.75cdefg	8.75cdefghi
C27	8.25defg	6.0f	6.0d	6.0i	11.25bcd	6.0j
C10a	8.25defg	7.5defgh	8.0bcd	7.0i	10.5bcde	11.0cdefg
C46	8.0efg	9.5bcdefg	8.0bcd	9.5efgh	6.0i	11.0cdefg
C30b	7.75efg	6.25fg	7.0cd	7.5hi	7.75ghi	7.75hij
L3	7.75efg	8.75bcdefgh	8.75bcd	6.0i	10.0bcdef	9.75cdefgh
C2	7.5efg	11.25bc	10.25b	9.0efgh	6.0i	6.0j
P1b	7.25fg	6.25fg	7.7cd	6.0i	10.0bcdef	9.25cdefghi
C34	7.0fg	8.5bcdefgh	6.0d	11.5cd	6.0i	6.0j
L1	6.75g	7.0defgh	7.0cd	13.25b	11.5bcd	7.5hij
C25	6.5g	6.5efh	6.0d	10.25defg	6.0i	9.0cdefghi

Mean LSD CV 9.162

3.125

1.986 8.537

8.53

1.986 7.373

2.455

1.986 9.796

1.715

1.986 9.105

2.021

1.986 10.073

2.743

1.986

aMeans followed by the same letters are not significantly different at 5% probability level. Significant means were separated using Least Significant Difference at α= 0.05 in SAS version 9.4. Note: The same letters (a, b, c, d, e, f, g, h, i, j,) within a row are not significantly different. C=starch casein, L=Luria Bertani, P1 and P4=ISP-project media

The mean value for antibacterial activity of study isolates against Staphylococcus aureus ranged from 6.5 mm to 12.25 mm in diameter. Isolate C52 (12.25mm) showed the highest antibacterial activity compared to isolate C25(6.75mm) which had the least inhibition activity. All the isolates under study had antibacterial activity against S. aureus.

The mean value for the inhibition zone for E. coli ranged from 6.25 mm to 8.75 mm. However, isolate L3 (8.75 mm) registered the highest inhibition activity against the test organism (E. coli). There was a statistically significant difference ($p>0.05$) in the isolates under study.

There was a highly significant difference ($p>0.05$) among antagonistic isolates against Listeria monocytogenes. Isolate L6(16.23mm) showed the highest antagonistic activity against L. monocytogenes compared to the standard; streptomycin (16.3mm). Moreover, L. monocytogenes showed resistance to

isolates P1b, L3, and L14(6.0mm). The difference among the isolates was statistically significant ($p>0.05$). The isolates C52, L14, C43, L6, and C8 exhibited the highest activity against S. aureus, R. planticola, V. furnissii, L. monocytogenes, and S. mitis respectively). These isolates were isolated from; chicken waste (C52, L14), waste dumpsite soil (C43), and cultivated soil (L6; C8). Isolate L3 that showed the highest activity against E. coli was isolated from cow dung waste followed by isolate C3 isolated from cultivated soil.

Molecular Characterization of the Bioactive Isolates

The 16S rRNA gene sequences of Actinomycetes from this study were aligned with sequences with similarity indices above 97% retrieved from the NCBI gene bank (https://www.ncbi.nlm.nih.gov), and identified up to species or genus level. The Neighbour Joining phylogenetic tree (Figure 2) built from the combined sequences showed that isolates 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 Pxiv 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. The evolutionary distances (Table 4) were computed using the Maximum Composite Likelihood method (Tamura et al., 2004).

DISCUSSION

The study was aimed to isolate Actinomycetes from forest, dumpsite, cultivated soil, farm yard manure and deforested regions and screen them for the production of secondary metabolites. Many studies have been done where Actinomycetes have been isolated from different places including soil, mountains, swamps and the marine environment (Guo et al., 2015; Kumar et al., 2013; Rao et al., 2012; Shakthi & Murugan, 2012). This has yielded good results where antibiotics against different gram positive and gram negative bacteria have been extracted. Pre-treatment of the samples with calcium carbonate and air drying them was done to enhance the rate of isolation and also inhibited the growth of common microbial species, allowing less abundant bacteria to be recovered. Antibiotics, nystatin (25 mg/mL) to reduce fungal contamination, and ampicillin (25 mg/mL) to eradicate gram-negative bacteria, were added to the selective media (Alferova

et al., 1989). Sambamurthy and Ellaiah (1974) employed amphotericin B as an antifungal drug in a technique similar to this.

The results showed more isolates were recovered from starch casein agar compared to other media used in the study. According to Kim (2016), starch casein agar (SCA) has a high carbon-to-nitrogen ratio and contains refractory complex carbons like starch and casein, making it ideal for Actinomycetes separation because these organisms can use high molecular weight polymers. Actinomycetes isolated in International Streptomyces media (ISP-1 and ISP-4) were shown to be slow-growing, according to the study. Actinomycetes were isolated on the same media by Anderson and Wellington (2001), and their colonies were slow-growing, aerobic, glabrous or chalky, folded, and with aerial and substrate mycelia of various colours.

The majority of the isolates were more active against gram-positive bacteria than gram-negative bacteria, according to antibacterial activity results. The findings in this study were comparable to those reported by Ganesan et al. (2017) and Gebreyohaness et al. (2013), who found that isolated Actinobacteria strains have antibacterial activity against several bacterial test strains. Furthermore, the observed difference in susceptibility of Gram-positive and Gram-negative bacteria test organisms could be attributed to the strains' collective secretions of diverse metabolites endowing them with broad-spectrum action (Benhadj et al., 2019). In addition, the majority of the isolates were ineffective against

E. coli. The findings of this study are comparable to those of Oskay et al., 2004 and Anasniriwittana et al., 2006. However, they were more active against S. aureus.

Actinomycetes molecular identification was successfully done through amplification of the 16S rRNA gene. The amplified region of 16S rRNA helped in the identification of Streptomyces strain (Cook and Meyer, 2003). According to literature, Actinomycetes species, 16 S rRNA gene are about 1250 base pairs while Streptomyces have 600 base pairs confirmed by actinomycetes specific primers and amplification of 16 S rRNA gene (Green and Barnes, 2010). Analysis of 16S-rRNA gene sequence amplification has been demonstrated to be a potential method for microorganism phylogeny investigation. It is reported that 16S rRNA gene sequencing provides genus identification in most cases more than 90% (Guo et al., (2008). For the identification of novel strains, 16S rRNA sequence method is most prevalently used due to its presence in almost all bacteria and the function of 16S rRNA gene over time has not changed (Janda and Abbot, 2007).

The phylogenetic analysis of the 16srRNA gene showed that the isolates were members of the Actinomycetes group. The similarity values were between 84% and 100%. There was therefore no diverse group of Actinomycetes found in the present study. A previous study on soil samples from a reserved area in Kenya reported similar results (Nonoh et al., 2010) where five isolates with antimicrobial activity clustered with known Streptomyces spp. In the present study, only eight isolates were able to be amplified by Actinomycetes-specific primers used. This could be attributed to the fact that the isolates that were not amplified could belong to the rare genera of Actinomycetes and in the future, more pairs of primers should be used including genera-specific primers.

The NCBI-BLASTn analysis result indicate that the sequence of isolate P4i showed 84% sequence similarity with Streptomyces celluloflavus. The species produces aurethricin which has the ability to degrade cellulose (Blunt et al., 2008) and its role in pharmaceuticals is the production of poly amino acids (Shih et al., 2004). The isolates C10 and C3 showed 99% sequence similarity with Streptomyces eurocidicus. The strain produces Azomycin, eurocidin C, D, E, tertiomycin A and B and 2-nitroimidazole. Isolates P1b and C10b had 100% sequence similarity with Streptomyces crystallinus. The species produces hygromycin-B which is an aminoglycoside antibiotic that kills, bacteria, fungi, and higher eukaryotic cells by inhibiting protein synthesis (Afifi et al., 2012). The antibacterial activity of Hygromycin A (HA) arises from protein synthesis inhibition and is dependent upon a methylenedioxy bridged - aminocyclitol moiety.

CONCLUSION

Actinomycetes are one of the most prominent components of soil microbial communities, and they play an important role in the generation of antibacterial secondary metabolites. Actinomycetes were isolated

successfully from soil samples taken in Meru South sub-county, Kenya. The selected Actinomycetes isolated from soil samples in were found to have antibacterial activity against the test pathogens. The

molecular characterization of the eight isolates with antibacterial activity showed they belonged to Streptomyces spp. Bioactive metabolites from these Streptomyces sp. have to be purified and minimum inhibition concentration analysis of the isolates has to be done for discovering novel compounds which have commercial value. Further studies on these are underway.

RECOMMENDATIONS

1. New techniques should be used for isolation of those taxa that cannot be isolated by conventional methods. Metagenomics can be employed hence those taxa that cannot be cultured can be captured.

2. There is need for isolating Actinomycetes from other unique environment in Kenya.

REFERENCES

Adegboye, M. F., & Babalola, O. O. (2016). Isolation and Identification of Potential Antibiotic Producing Rare Actinomycetes from Rhizospheric Soils. Journal of Human Ecology, 56(1/2), 31–41.

Afifi M.M., Atta H. M, Elshanawany A. A., Abdoul-raouf , U.M and El-Adly, A.M., (2012). Biosynthesis of Hygromycin-B Antibiotic by Streptomyces crystallinus AZ151 Isolated from Assuit, Egypt Bacteriology Journal, 2: 46-65

Alferova, I., Terekhova, L., Prauzer, K. (1989). Selective medium with nalidixic acid for Isolating antibiotic-producing Actinomycetes Antibiotics Khimioter, 34: 344-348

Anansiriwattana, W., Tanasupawat, S., Amnuoypol, S. and Suwanborirux, K. (2006). Identification and antimicrobial activity of Actinomycetes from the soil in Samed island, and geldanamycin from strain PC4-3. The Thai Journal of Pharmaceutical Science. 30: 49-56.

Anderson, A. S., Wellington, E. M.H. (2001). The taxonomy of Streptomyces and related genera.

International Journal of Systematic and Evolutionary Microbiology 51: 797-814.

Barkaa, E. A, Vatsaa, P, Sancheza, L, Gaveau-Vaillanta N, Jacquarda, C. & Klink, H. P (2016). Taxonomy, physiology, and natural products of Actinobacteria. Microbiology and Molecular Biology Review: 80(1):1-43

Baniya A., Singh S., Singh M., (2019). Isolation and screening of antibiotics producing Streptomyces spp from the soil collected around the root of Alnus nepalensis from Godawari. Nepal Journal of Biotechnology.6(1):46–56.

Bizuye, A, Moges, F, & Andualem, B. (2013). Isolation and screening of antibiotic-producing Actinomycetes from soils in Gondar town, North West Ethiopia. Asian Pacific Journal of Tropical Disease. 31;3 (5):375-81

Chamikara, P. (2016). Advanced Study on selected taxonomic groups of Bacteria and Archae Actinomycetes. 01-09. http://www.academia. edu/26067743/ Actinomycetes

Clarridge, J. E. I. (2004). Impact of 16S rRNA Gene Sequence Analysis for Identification of Bacteria on Clinical Microbiology and Infectious Diseases. Clinical Microbiology Reviews, 17(4), 840–862.

Chen, X., Jiang, Y., Li, Q., Han, L., & Jiang, C. (2017). Molecular Phylogenetic Identification of Actinobacteria Xiu. In Actinobacteria - Basics and Biotechnological Applications (Vol. 56, pp. 141–174).

Cook, A. E, Meyers P. R. (2003). Rapid identification of filamentous actinomycetes to the genus level using genus-specific 16S rRNA gene restriction fragment patterns. International journal of systematic and evolutionary microbiology.;53(6):1907-15.

Davies, F.L., Williams, S.T. (1970). Studies on the ecology of Actinomycetes in soil I. The occurrence and distribution of Actinomycetes in pine forest soil. SoilBiology and Biochemistry. 2, 227-238.

Eilers, K. G., Debenport, S., Anderson, S., & Fierer, N. (2012). Digging deeper to find unique microbial communities: the strong effect of depth on the structure of bacterial and archaeal communities in soil. Soil Biology and Biochemistry, 50, 58–65

Fierer, N., Schimel, J. P., & Holden, P. A. (2003). Variations in microbial community composition through two soil depth profiles. Soil Biology and Biochemistry, 35, 167–176

Fguira L. F., Fotso S, Ameur-Mehdi R. B, Mellouli L. L., (2005): Purification and structure elucidation of antifungal and antibacterial activities of newly isolated Streptomyces sp. Strain US80. Research in Microbiology.

Ganesan P., David R. H. A., Reegan A. D., (2017). Isolation and molecular characterization of Actinomycetes with antimicrobial and mosquito larvicidal properties. Beni-Seuf University Journal of Basic and Applied Sciences. 6(2):209–217.

Goodfellow, M., Williams, S.T. (1983). Ecology of Actinomycetes. Annual Review of Microbiology. 37, 189-216.

Gebreyohannes G, Moges F, Sahile S, Raja N (2013). Isolation and characterization of potential antibiotic- producing Actinomycetes from water and sediments of Lake Tana, Ethiopia. Asian Pacific Journal of Tropical Biomedicine 3:426–435.

Guo, X., Liu, N., Li, X., Ding, Y., Shang, F., Gao, Y., Ruan, J. & Huang, Y. (2015). Red Soils Harbour Diverse Culturable Actinomycetes that are promising sources of novel secondary metabolites. Applied and Environmental Microbiology, 81, 3086-3103.

Hopwood, D. A., and Wright, H. M. (1973). A plasmid of Streptomyces coelicolor carrying a chromosomal locus and its inter-specific transfer. Journal of Genetic Microbial. 79, 331–342.

Kuster, E., Williams, S. (1964). Selective media for the isolation of Streptomyces. Nature 202: 928-929.
Lakshmi, V., & Roshmi, D. (2014). Extracellular synthesis of silver nanoparticles by the Bacillus strain

CS 11 isolated from the industrialized area, 4, 121–126.

Messaoudi, O., Bendahou, M., Benamar, I., & Abdelwouhid, D. E. (2015). Identification and preliminary characterization of non-polyene antibiotics secreted by a new strain of actinomycete isolated from sebkha of Kenadsa, Algeria. Asian Pacific Journal of Tropical Biomedicine, 5(6), 438–445.

Mohseni, M., Norouzi, H., Hamedi, J., Roohi, A. (2013). Screening of Antibacterial Producing Actinomycetes from Sediments of the Caspian Sea. International Journal of Molecular Cell Medical. 2(2): 64-71

Nonoh, J. O., Lw, W., Masiga, D., Herrmann, R., Presnail, J. K., Schepers, E., .& Boga, H. I. (2010). Isolation and characterization of Streptomyces species with antifungal activity from selected national parks in Kenya. African Journal of Microbiology Research, 4(9), 856-864.

Orooba, M. F., Ali, A. S., Khansa, M. Y, Gires, U., & Asmat, A. (2017). Isolation, screening, and antibiotic profiling of marine Actinomycetes extract from the coast of Peninsular Malaysia. International Journal of Chemistry and Techniques Reviews 3:212-224.

Oskay, M., Tamer, A. Ü. and Azeri, C. (2004). Antibacterial activity of some Actinomycetes isolated from farming soils of Turkey. African Journal of Biotechnology 441-446.

Panchagnula, B. & Terli, R. (2011). Screening of marine sediments from Bay of Bengal for isolation of Lipolytic Actinobacteria and characterization of the most potent isolates. International Journal of Biology 3:33-43

Pandey, A., Ali I, Butola KS, (2011). Isolation and characterization of Actinomycetes from soil and evaluation of antibacterial activities of Actinomycetes against pathogens. International Journal of Applied Biology and Pharmaceutical Technology.; 2(4):384-92

Pastrana, C., Suárez, Z., González-Acosta. A. (2016). Bioprospecting for actinobacterial with antimicrobial properties isolated from rivers colombia .1265-1259: 15. Tropical journal of pharmaceutical research

Pooja, S., Rajesh, K., Mahesh, S. Y, Nityan, M., & Dilip, K. A. (2015). Isolation and Characterization of Streptomyces with Plant Growth Promoting Potential from Mangrove Ecosystem. Poland Journal of Microbiology 64: 339-349.

Prescott, L. M, Harley J. P, Klein D. A (1991) Microbiology, 2nd and. WMC Brown Publishers, New York, p 912

Sacchi, C. T., Whitney, A. M., Reeves, M. W., Mayer, L. W., and Popovic, T. (2002). Sequence Diversity of Neisseria meningitidis 16S rRNA Genes and use of 16S rRNA Gene sequencing as a Molecular Subtyping Tool. Journal of Cinical Microbiology, 40(12), 4520–4527

Saker l et, S Peter, Z Abdelghani, M Atika, B Noureddine, R Saker. (2015). Sahara Northern from isolated actinomycete halophilic novel .430-423: 70 Microbiology Current

Salim, F.M., Sharmili, S.A., Anbumalarmathi, J., Umamaheswari, K. (2017). Isolation, Molecular Characterization, and Identification of Antibiotic Producing Actinomycetes from Soil Samples. Journal of Applied Pharmaceutical Science. 7(9);069-075.

Sambamurthy R, Ellaiah P. A new Streptomycin producing neomycin (B and C) complex, Streptomyces marinensis (part-1). Hind Antibiotic Bull 1974; 17:24-8.

Sathi, Z. S, Md A. A Rahman, Gafur M. A., (2001). Identification and in vitro anti-microbial activity of a compound isolated from Streptomyces species. Pakistan Journal of Biological Science.; 4 (12): 1523-1525.

Shirling, E. B. and Gottlieb D. (1966). "Methods for characterization of Streptomyces species," International Journal of Systematic Bacteriology, volume 16, . 3, pp. 313–340

Singh, V., Haque, S., Singh, H., Verma, J., Vibha, K., Singh, R., Tripathi, C. K. M. (2016). Isolation, screening, and identification of novel isolates of actinomycetes from India for antimicrobial applications. Frontiers in Microbiology, 7 (1–9).

Sujatha, P., & Swethalatha, P. (2017). Isolation and screening of novel Streptomyces from sediments of Bay of Bengal near Srikakulam coast. International Journal of Current Pharmacy Reviews 9: 40- 44.

Tamura, K., Nei, M., and Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences of the United States of America, 101(30), 11030-11035

Velayudham, S., and Murugan, K. (2012). Diversity and Antibacterial Screening of Actinomycetes.

Journal of Microbiology Research, 2(2), 41–46

Williams, S. T, Neilly T, Wellington E. M. H (1977). The decomposition of vegetation growing mineral mine waste. Soil Biology and Biochemistry: 9: 271-275

Williams S. T., Good fellow M., Alderson G. (1989). Bergey's manual of systematic bacteriology, in Genus Streptomyces Waksman and Henrici 1943, 339AL, Vol. 4, eds Williams S. T., Sharpe M. E., Holt

J. G., editors. (Baltimore, MD: Williams and Wilkins;), 2452–2492

Zainal Abidin, Z. A., Abdul Malek, N., Zainuddin, Z., & Chowdhury, A. J. K. (2016). Selective isolation and antagonistic activity of Actinomycetes from mangrove forest of Pahang, Malaysia. Frontiers in Life Science, 9(1), 24–31.

Zhang, Z.; Pang, X.; Xuewu, D.; Ji, Z.; Jiang, Y. (2005). Role of peroxidase in anthocyanindegradation in litchi fruit pericarp. Food Chemistry. 90, 47–52.